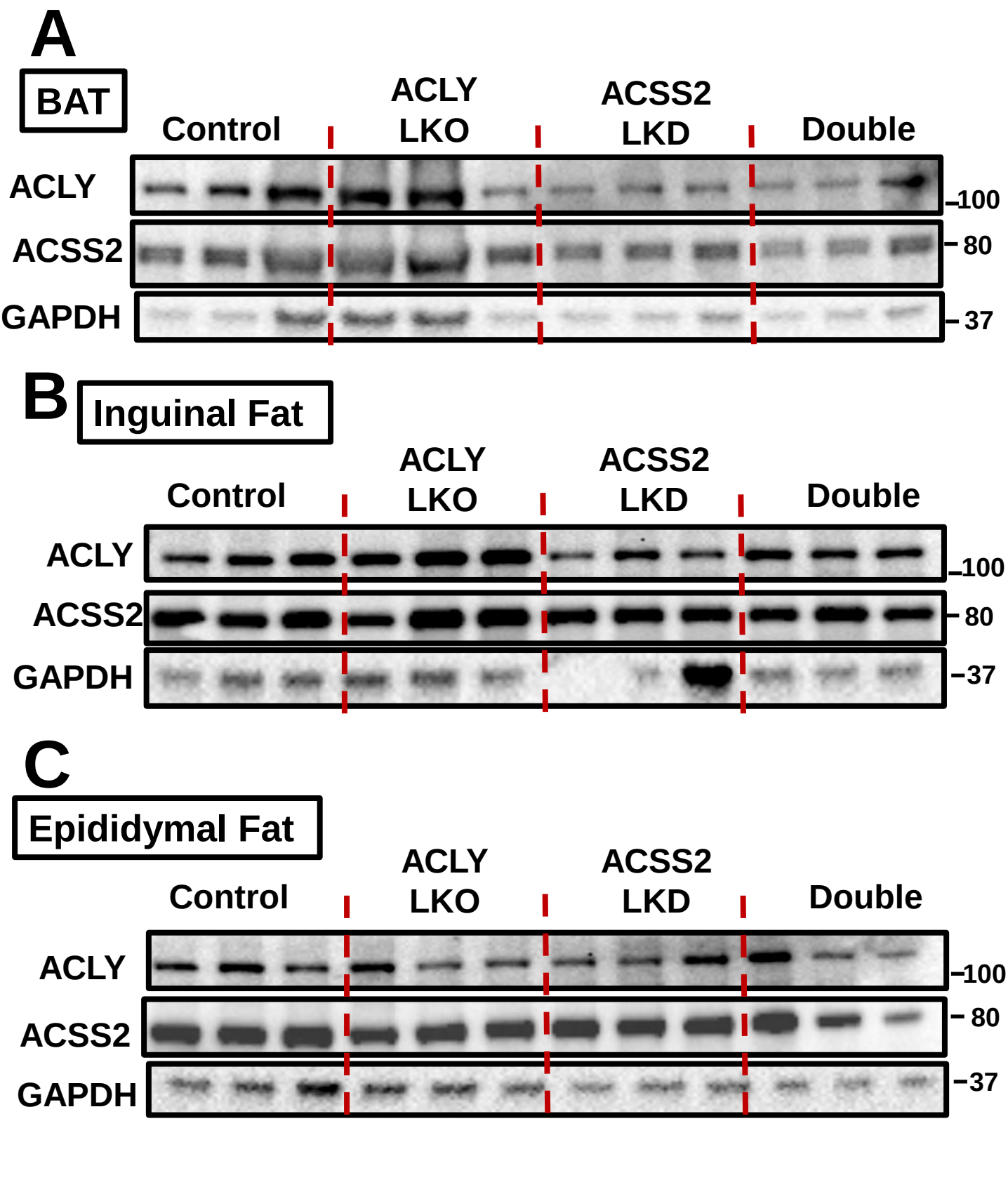


Figure S1: Confirmation of liver specific targeting of the AAV constructs: Immunoblots of ACLY and ACSS2 in **(A)** brown, **(B)** inguinal and **(C)** epididymal fat depots.

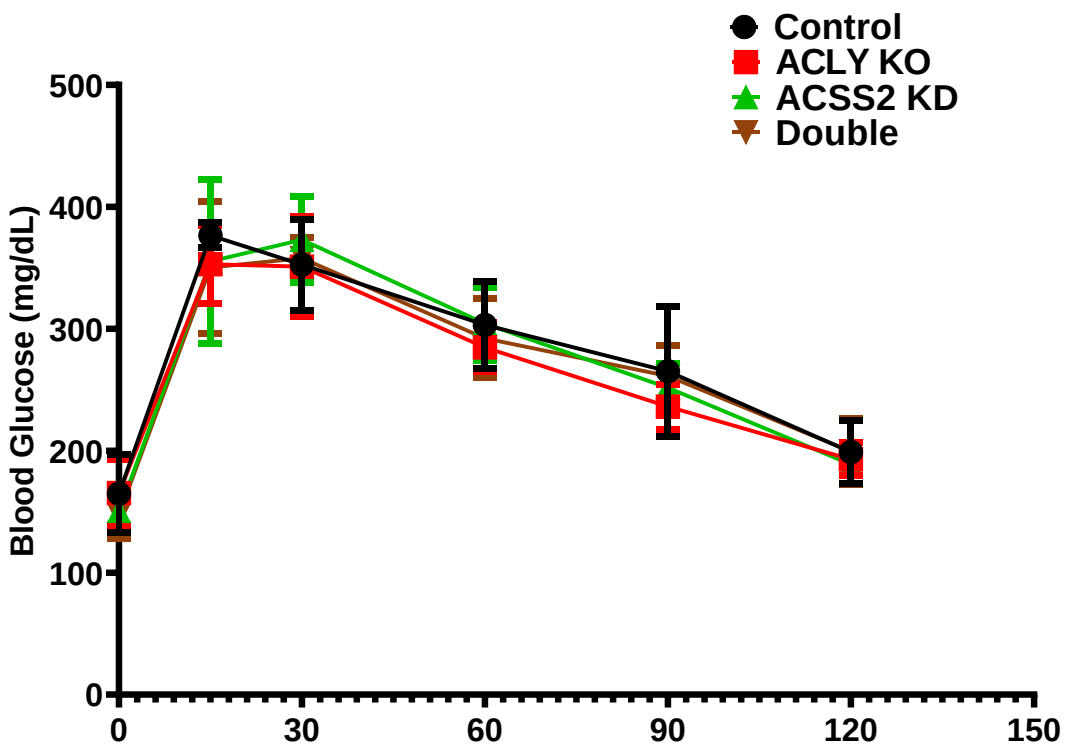


Figure S2: ACLY and/or ACSS2 depletion in HFD fed mice induced no changes in overall glucose tolerance.

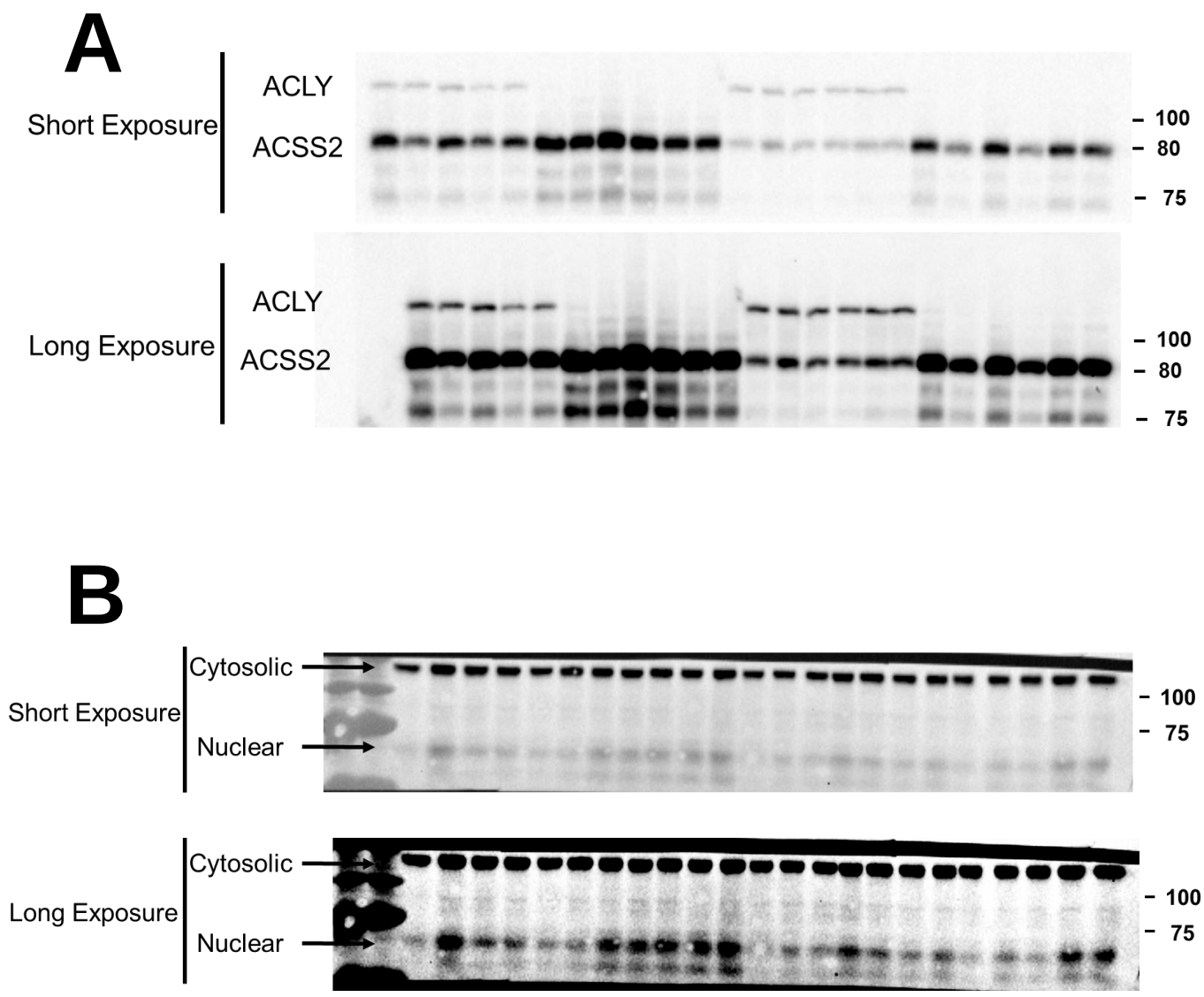
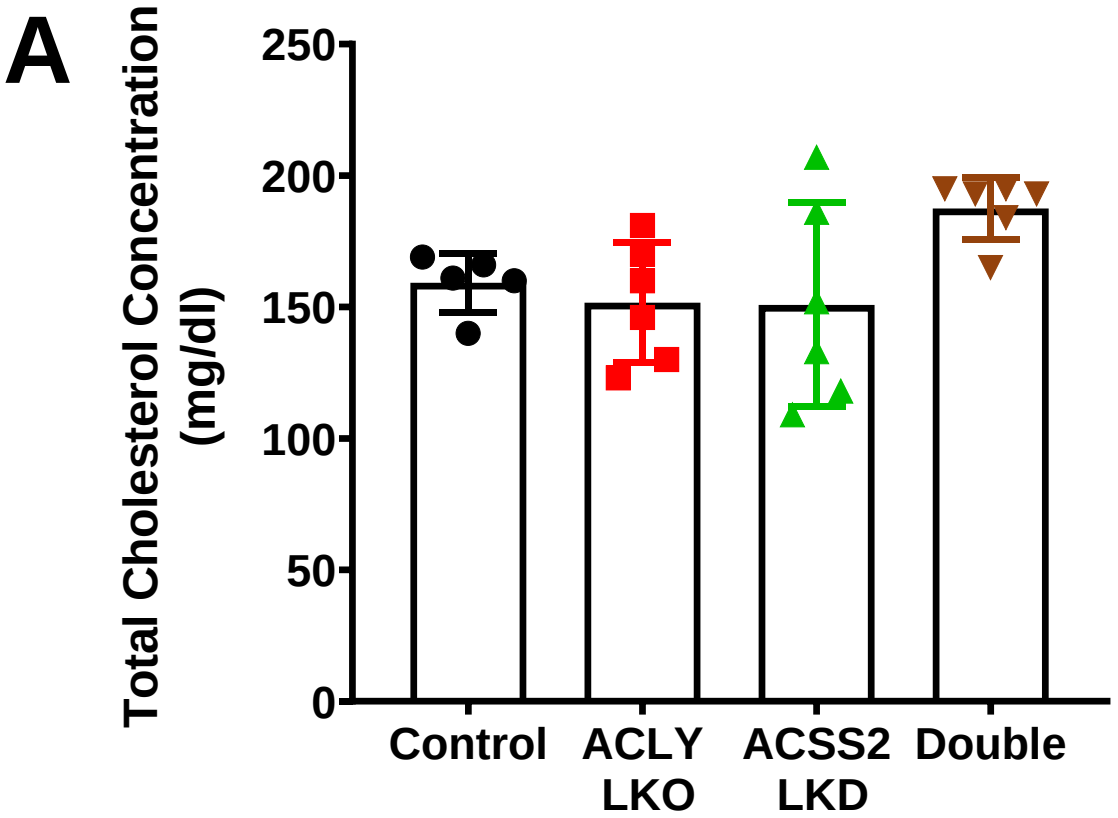
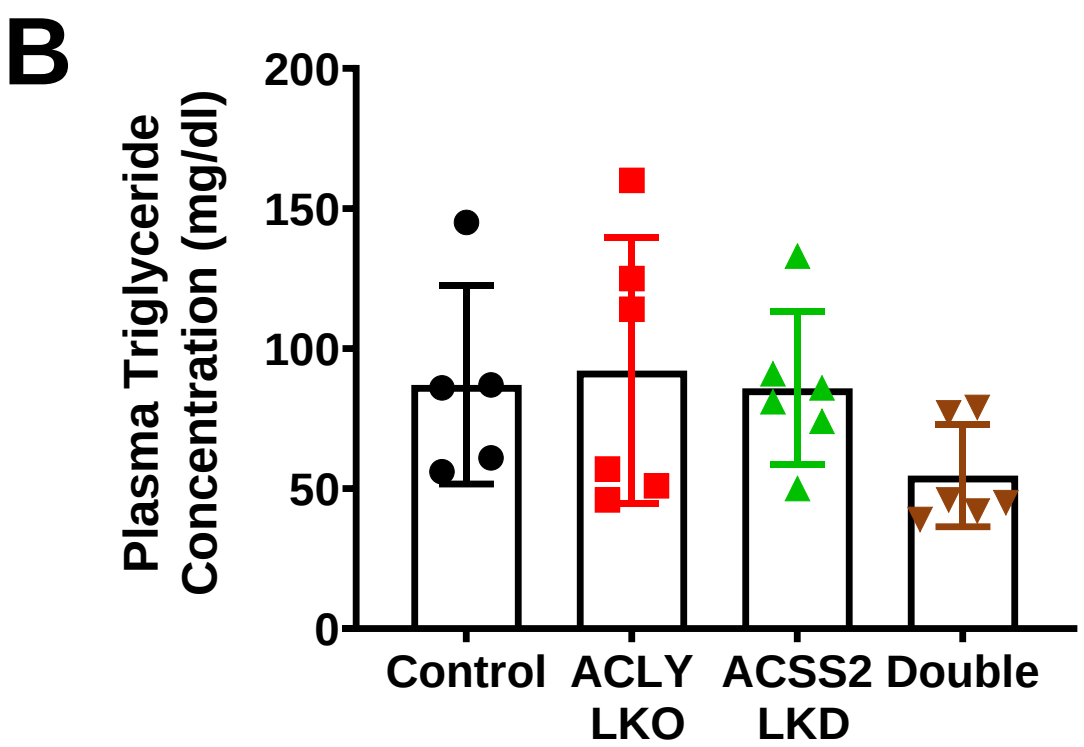


Figure S3: Raw data of (A) ACLY & ACSS2 blots in Fig. 2E and (B) SREBP1c western blots in Fig. 4B with short and long exposures





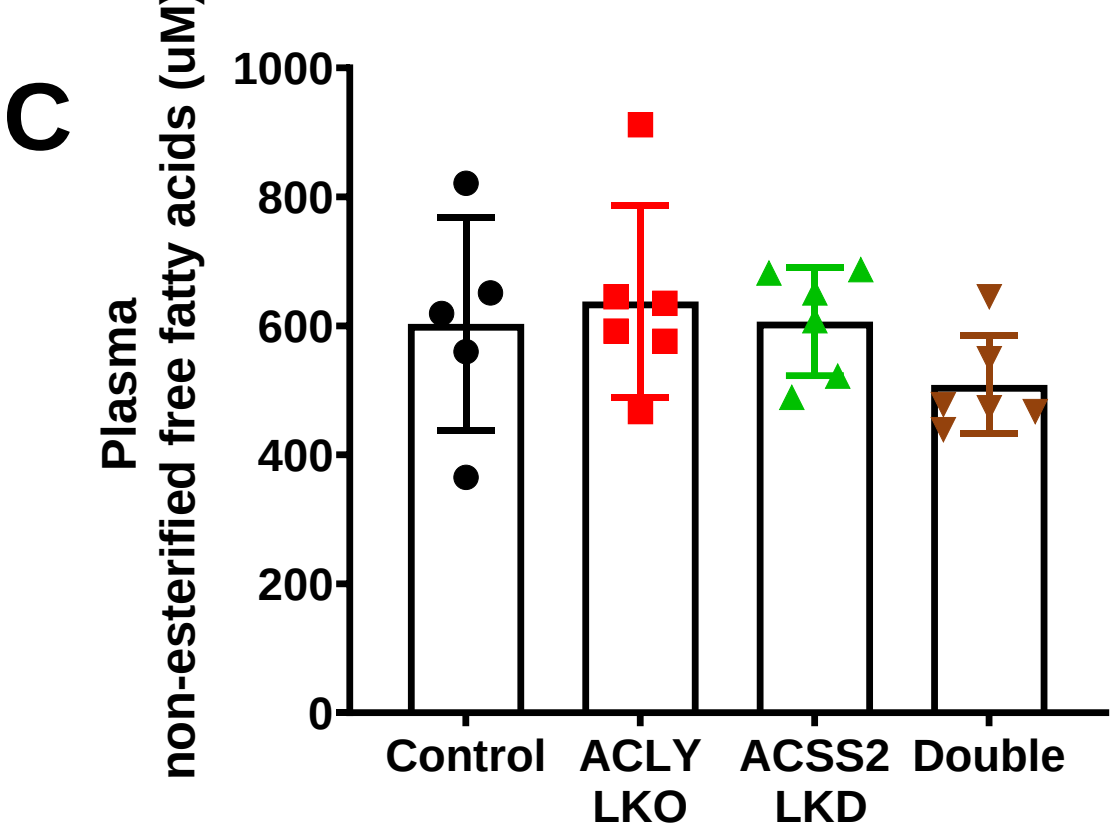


Figure S4: Liver depletion of ACLY, ACSS2 or double depletion does not change the plasma lipid profile in HFD fed mice. Plasma levels of **(A)** Total cholesterol, **(B)**TGs **(C)** Non esterified fatty acids.

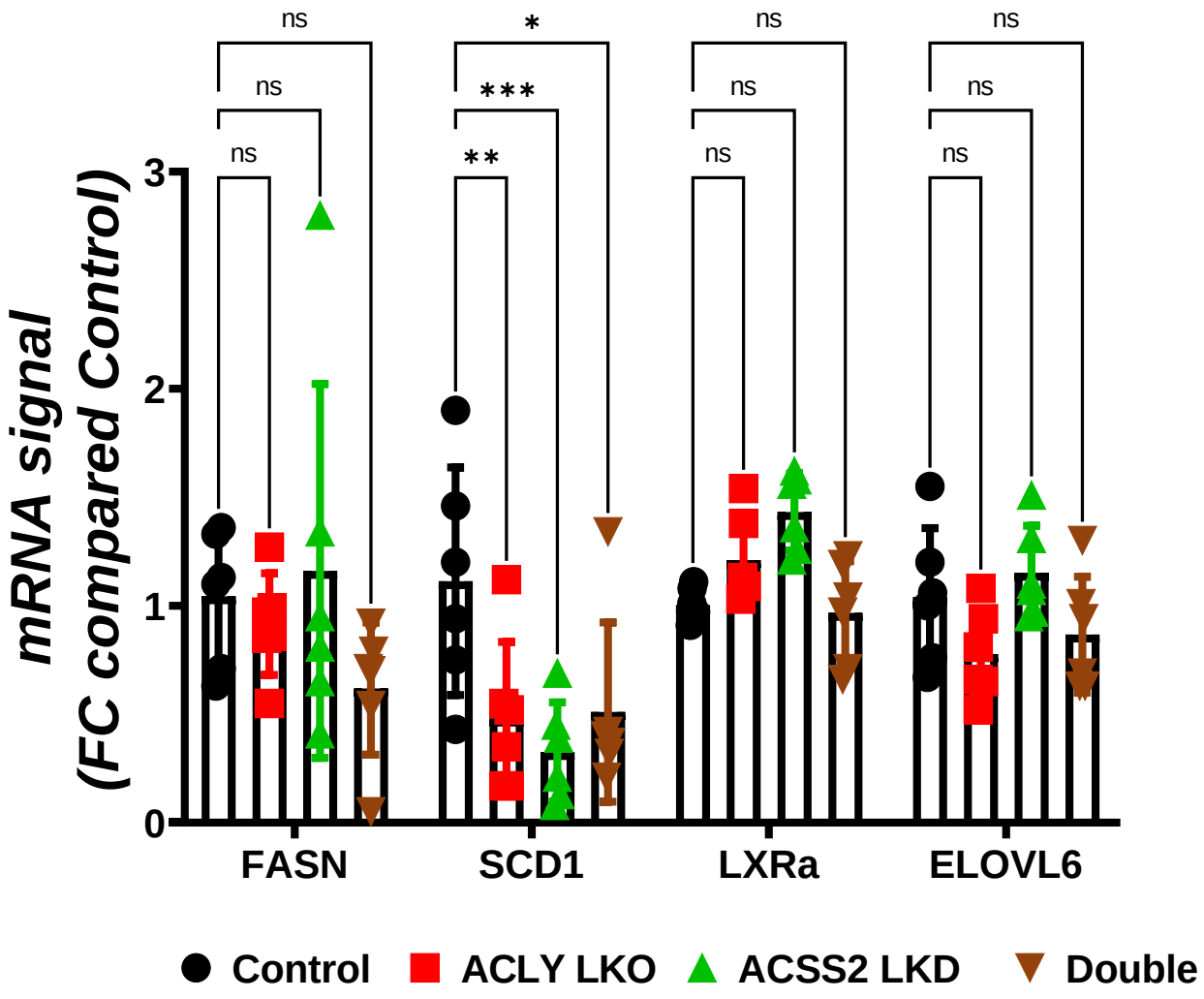


Figure S5: Liver depletion of ACLY, ACSS2 or double depletion does not induce an upregulation of DNL enzyme gene expression in CHOW fed mice.

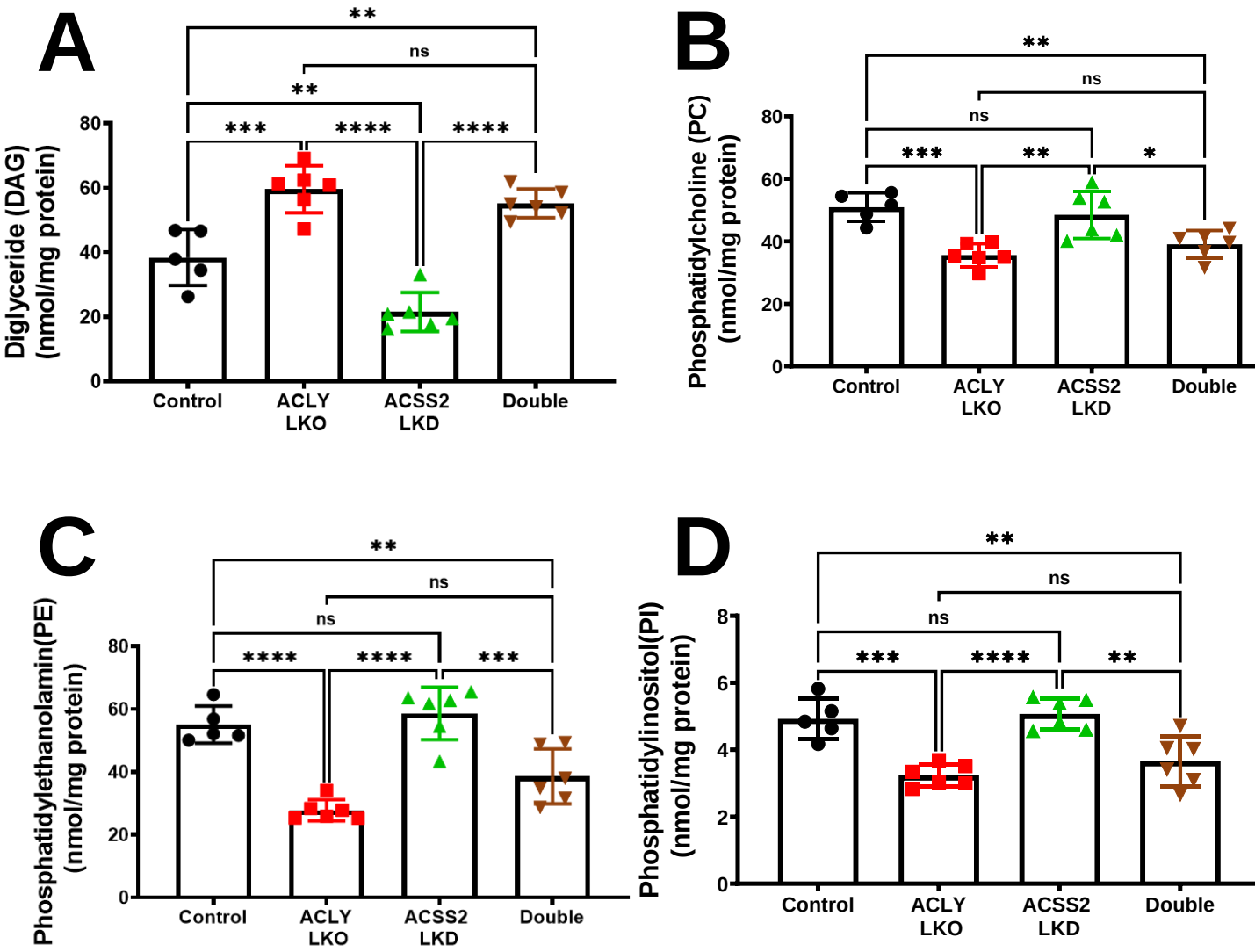
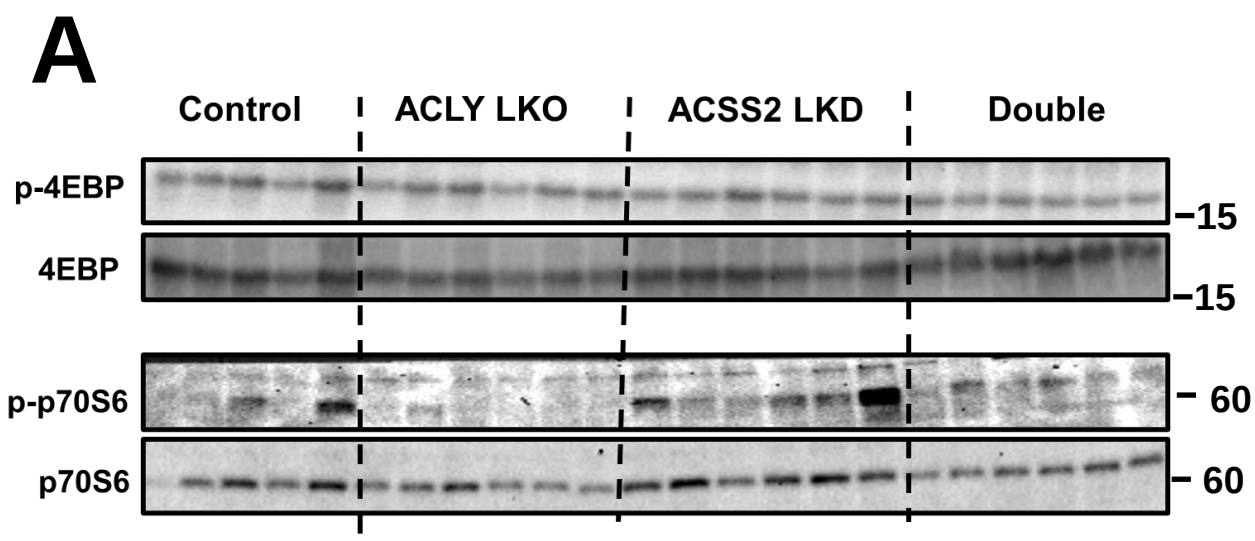
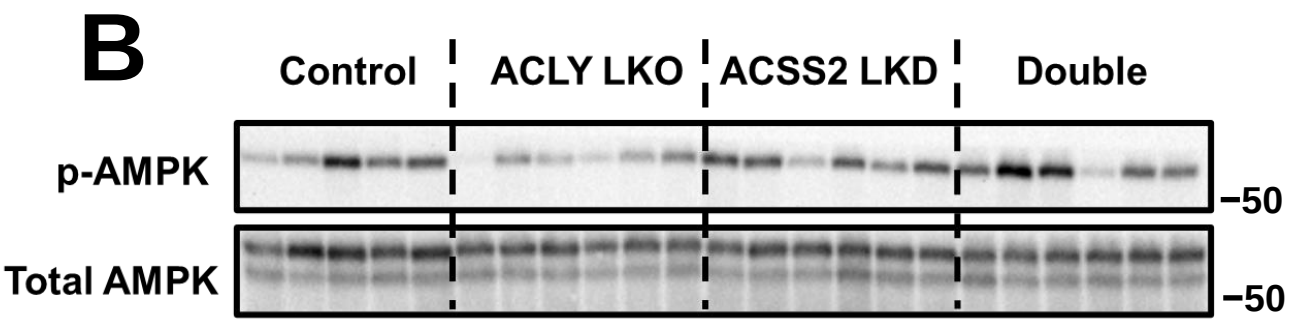


Figure S6: Lipidomics analysis demonstrates accumulation of DAGs in ACLY-depleted but not ACSS2-depleted livers, and inverse correlation with phospholipid biosynthesis. Liver **(A)** DAGs **(B)** Phosphatidylcholine **(C)** Phosphatidylethanolamine **(D)** Phosphatidylinositol levels in the livers of HFD fed groups. (ns: Not significant, *: p<0.05, **:p<0.005, ***:p<0.0005, ****:p<0.00005)





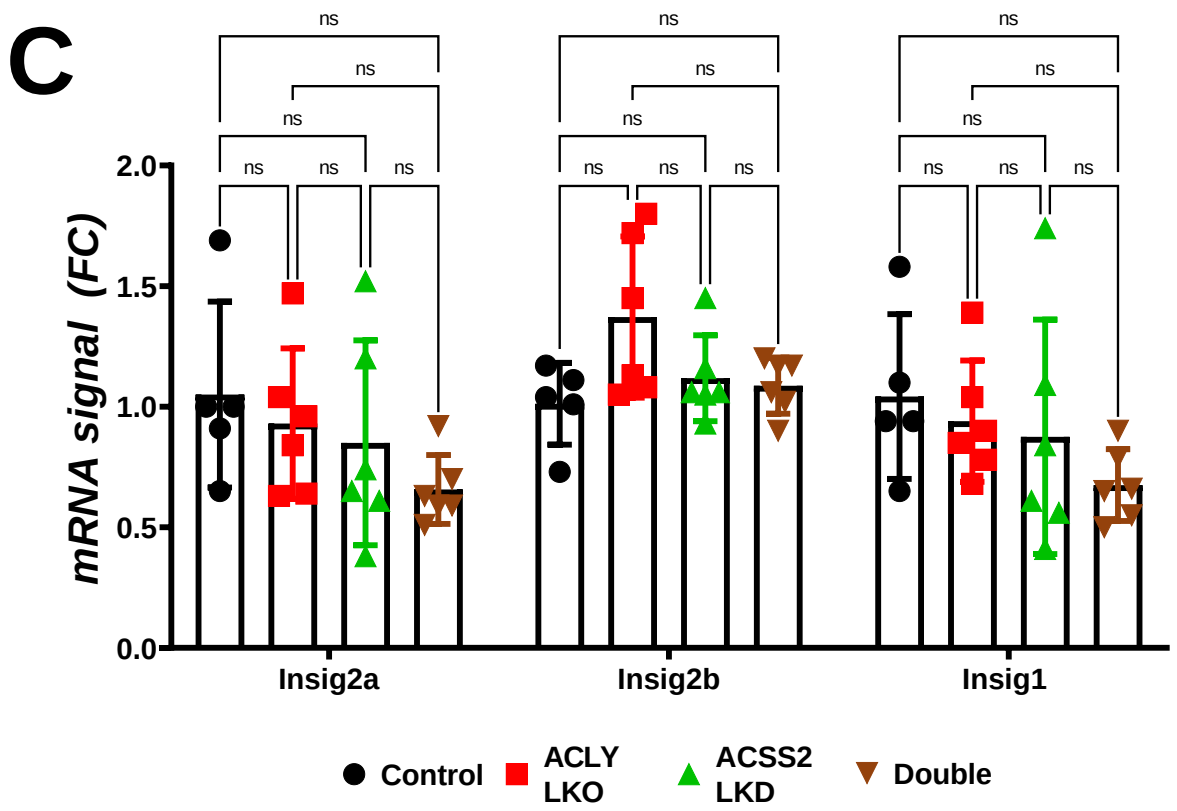


Figure S7: Increased Srebp1c processing in ACLY depleted mice in HFD is independent of (A) mTOR activation, (B) AMPK phosphorylation or (C) Insig pathways