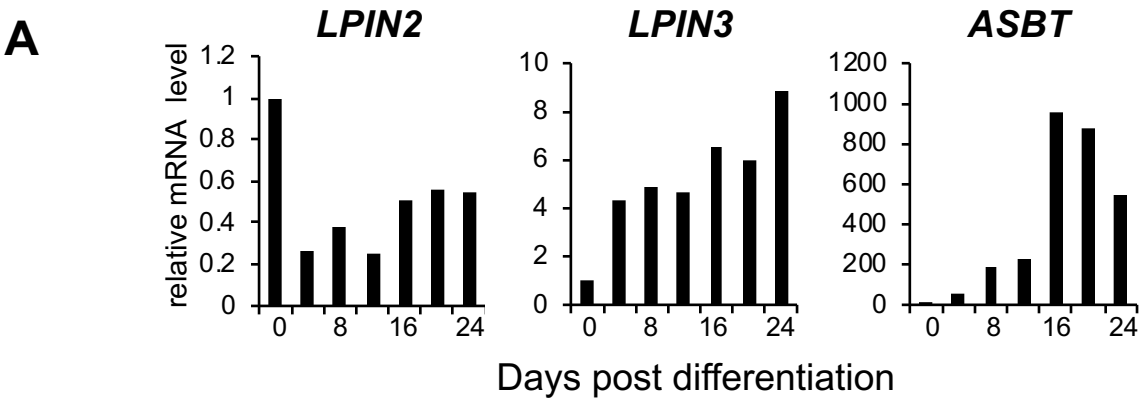
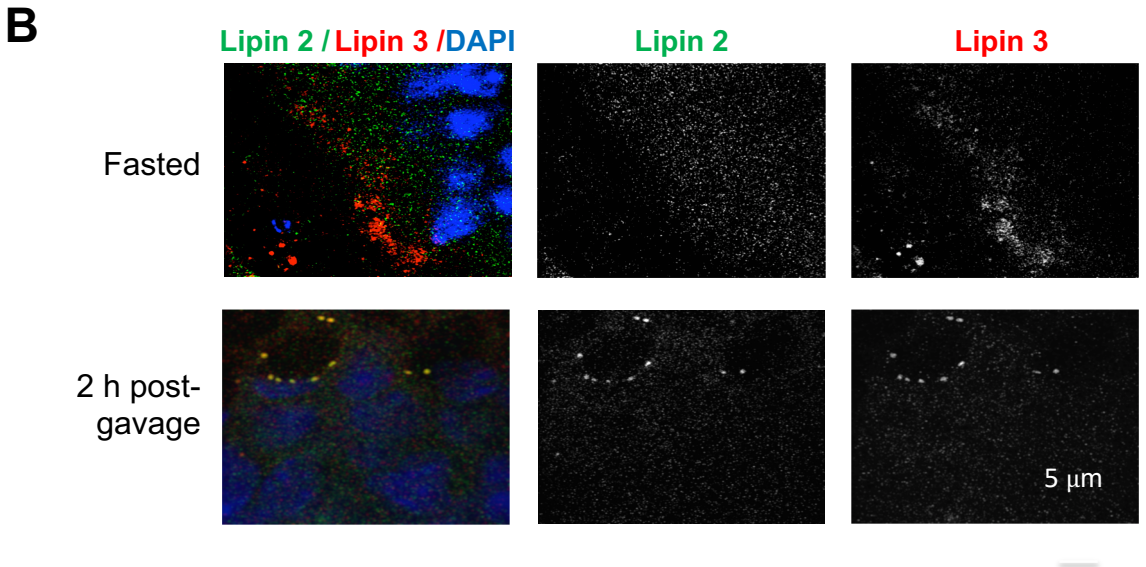
Fig. S1


A
LPIN2
LPIN3
ASBT
relative mRNA level
1.2
1
0.8
0.6
0.4
0.2
0
10
8
6
4
2
0
1200
1000
800
600
400
200
0
0
8
16
24
Days post differentiation


B
Lipin 2 / Lipin 3 /DAPI
Lipin 2
Lipin 3
Fasted
2 h post-gavage
5 µm

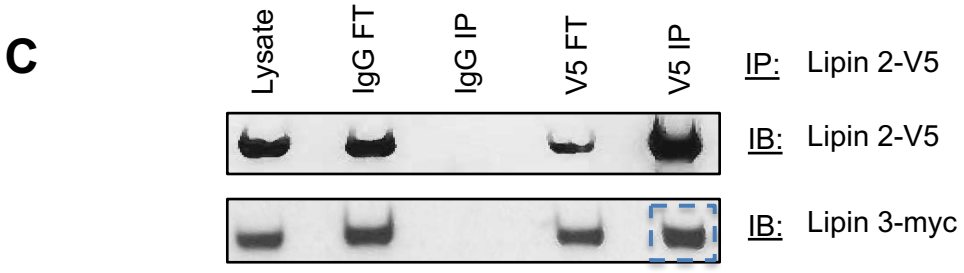

C
Lysate
IgG FT
IgG IP
V5 FT
V5 IP
IP: Lipin 2-V5
IB: Lipin 2-V5
IB: Lipin 3-myc

Figure S1. Lipin 2 and lipin 3 expression, subcellular localization, and co-immunoprecipitation.

(A) qPCR analysis of *LPIN2*, *LPIN3* and *ASBT* gene expression analysis during Caco-2 cell differentiation performed by qPCR,. n = 3.

(B) Representative fluorescence image showing localization of endogenous lipin 2 (green) and lipin 3 (red) in proximal small intestine of mice 2 hr after gavage with olive oil containing BODIPY-labeled FA. Blue, DAPI.

(C) Co-immunoprecipitation of lipin 2 and lipin 3 proteins. Lipin 2-V5 and lipin 3-myc plasmids were co-expressed in HEK-293 cells, lipin 2-V5 and lipin 3-myc and immunoprecipitated using with V5 antibody or IgG control antibody, then lipin 2-V5 and lipin 3-myc were detected by immunoblotting. Dashed line highlights lipin 3 protein pulled down by with lipin 2 antibody.

Fig. S2

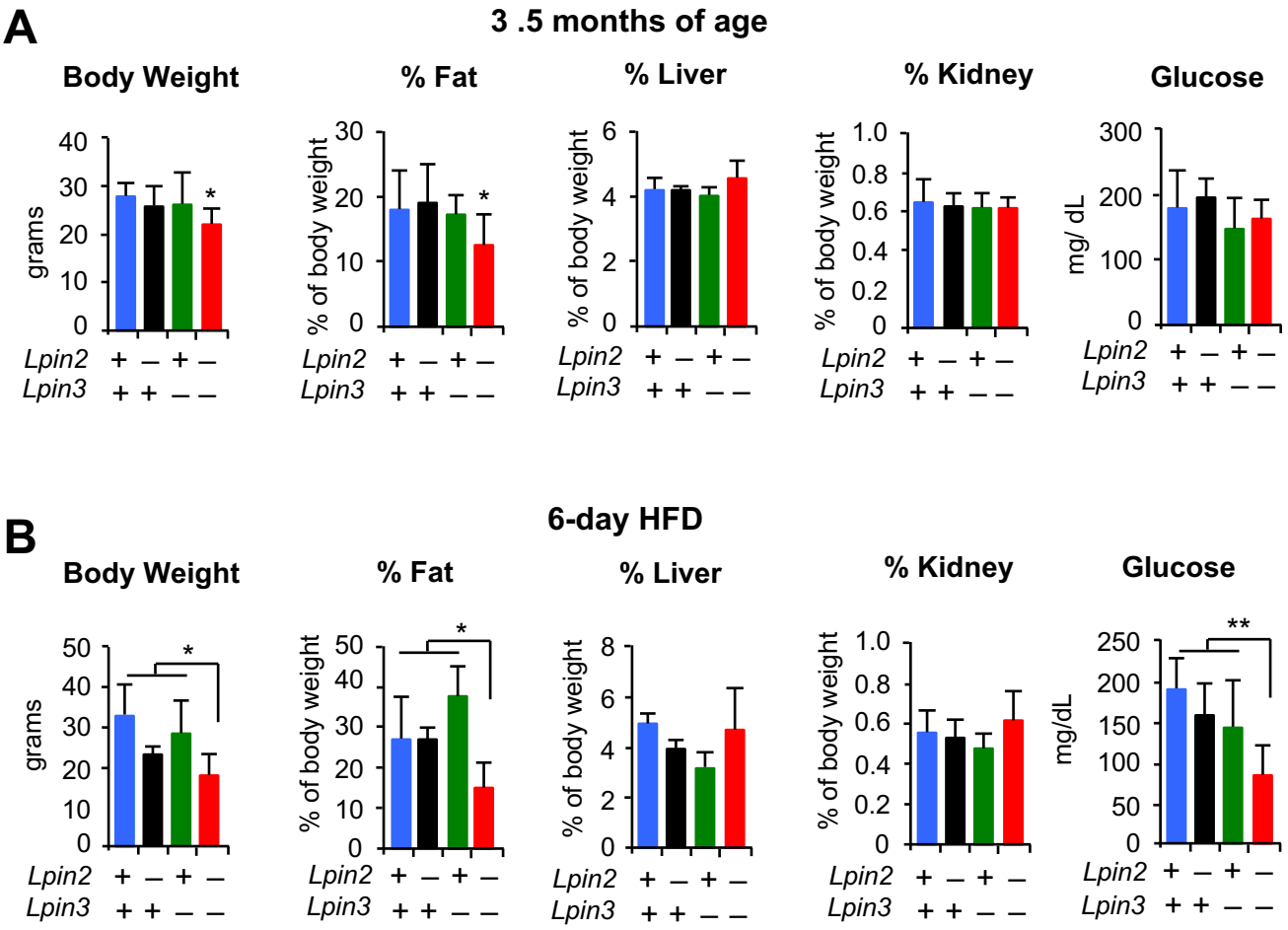


Figure S2. Tissue weights and *Lpin1* gene expression in *Lpin*2/3 KO mice.

*(A) Lpin*2/3 KO mice (3.5 months of age) have reduced body weight and fat mass, normal relative liver and kidney weight and glucose levels. n = 4–6; *, $p < 0.05$.

(B) Body weight, fat tissue weight, and fasting glucose levels are reduced in 5-month-old *Lpin*2/3 KO mice fed a high- fat diet for 6 days. n = 4–6; *, $p < 0.05$; **, $p < 0.01$.

Fig. S3

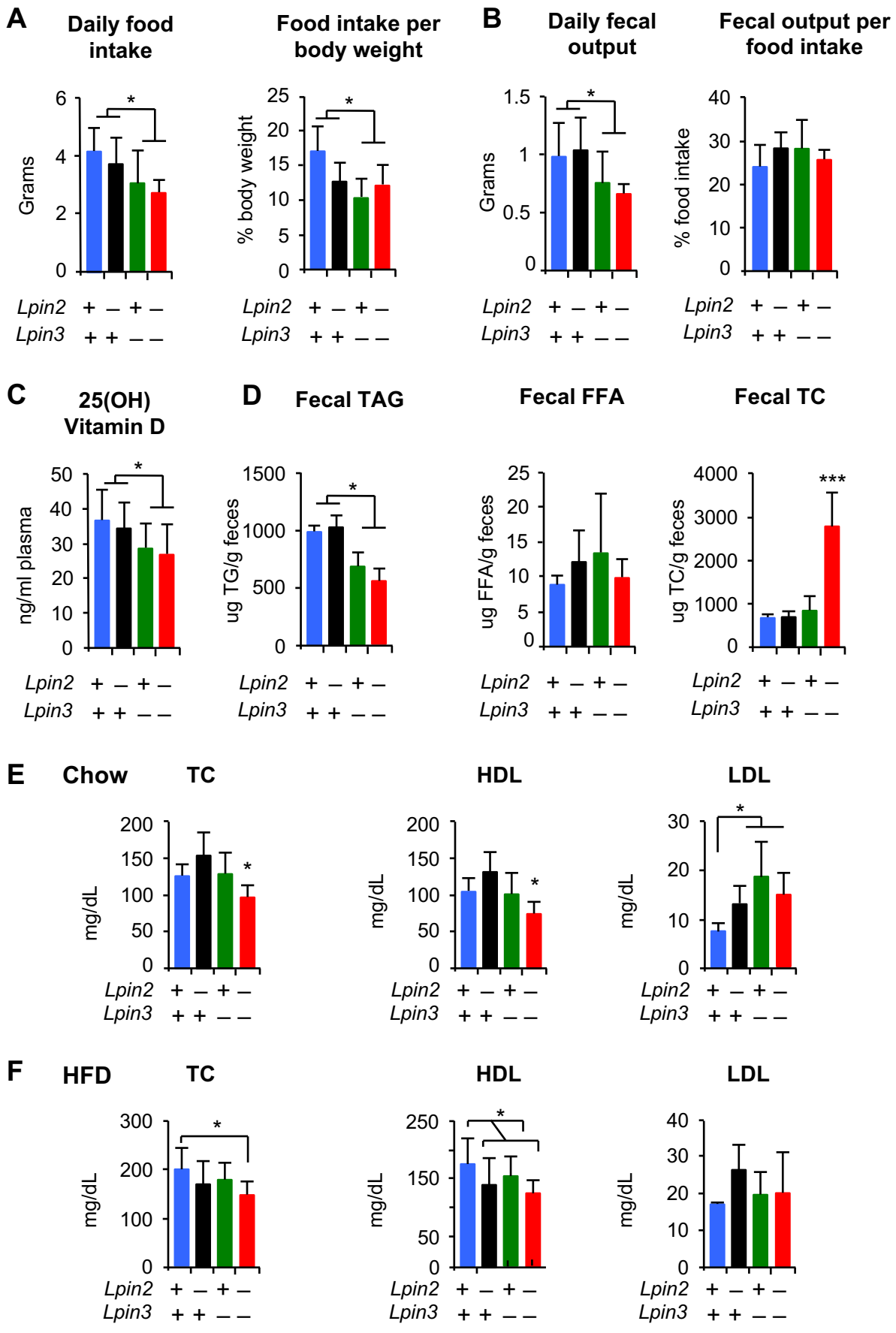

A
Daily food intake
Grams
Food intake per body weight
% body weight
B
Daily fecal output
Grams
Fecal output per food intake
% food intake
C
25(OH) Vitamin D
ng/ml plasma
D
Fecal TAG
ug TG/g feces
Fecal FFA
ug FFA/g feces
Fecal TC
ug TC/g feces
E
Chow
TC
HDL
LDL
mg/dL
F
HFD
TC
HDL
LDL
mg/dL
Lpin2 + – + –
Lpin3 + + – –

Figure S3. Food intake, nutrient absorption and lipoprotein levels in *Lpin*2/3 KO mice.

(A) Daily food intake in singly housed mice fed chow diet presented as raw values (left) or adjusted per body weight (right). n = 5; *, $p < 0.05$.

(B) Daily fecal output in mice from (A).

(C) Plasma vitamin D levels from mice in (A).

(D) Fecal lipid content from feces collected in (B).

(E) Plasma lipoprotein levels in mice fed chow diet (E) or high fat diet for 6 days (F). n = 4–6.

Fig. S4

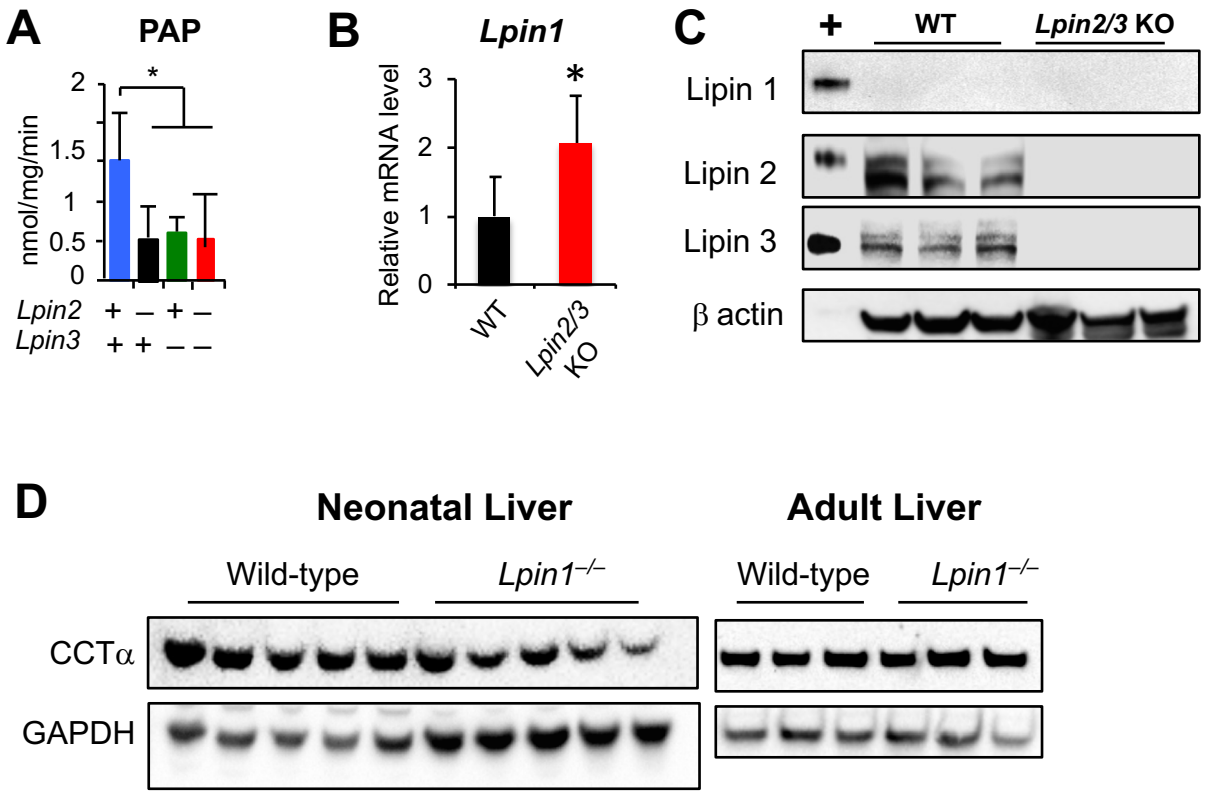


Figure S4. PAP activity and lipin 1 levels in lipin 2/3 KO intestine.

(A) PAP activity in duodenal lysates from mice of the four genotypes indicated. N = 4–6/genotype; *, $p < 0.05$, pair-wise comparison after significant 2-way ANOVA.

(B) Lpin1 mRNA levels in duodenum from wild-type (WT) and lipin 2/3 KO mice. N = 5/genotype.

(C) Lipin protein levels in wild-type (WT) and *Lpin*2/3 KO duodenum. +, recombinant lipin 1, lipin 2, or lipin 3 immunoblot control. N = 3/genotype.

(D) CCTα protein levels in lipin 1-deficient liver are not elevated during the neonatal period (7 days postnatally) nor in adult mice.

Fig. S5

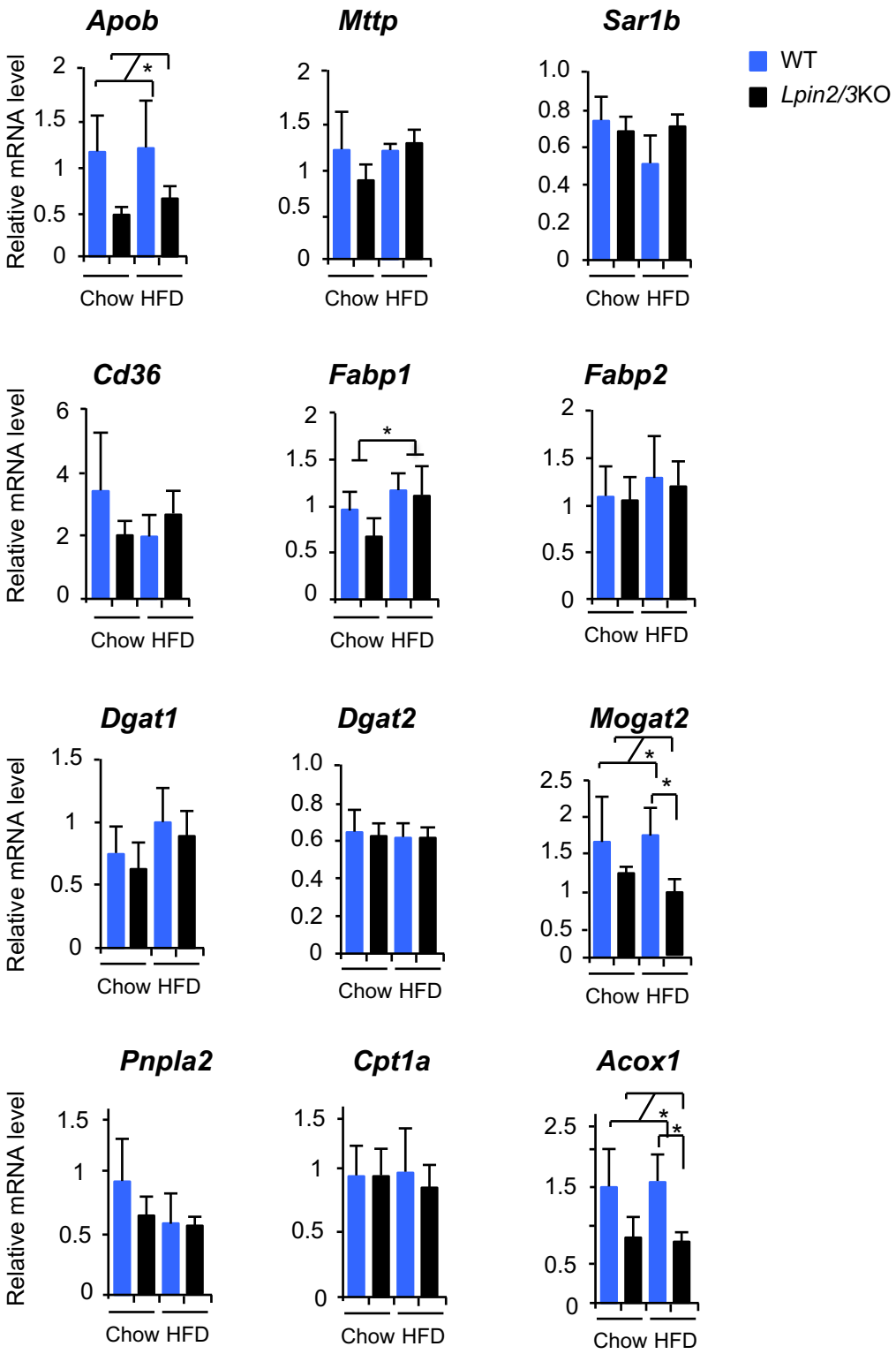


Figure S5. Gene expression in the proximal small intestine in mice fed chow or high-fat diet (HFD) for 6 days.

n = 4–6. *, $p < 0.05$; **, $p < 0.01$. Analyzed by 2-way ANOVA.

Fig. S6

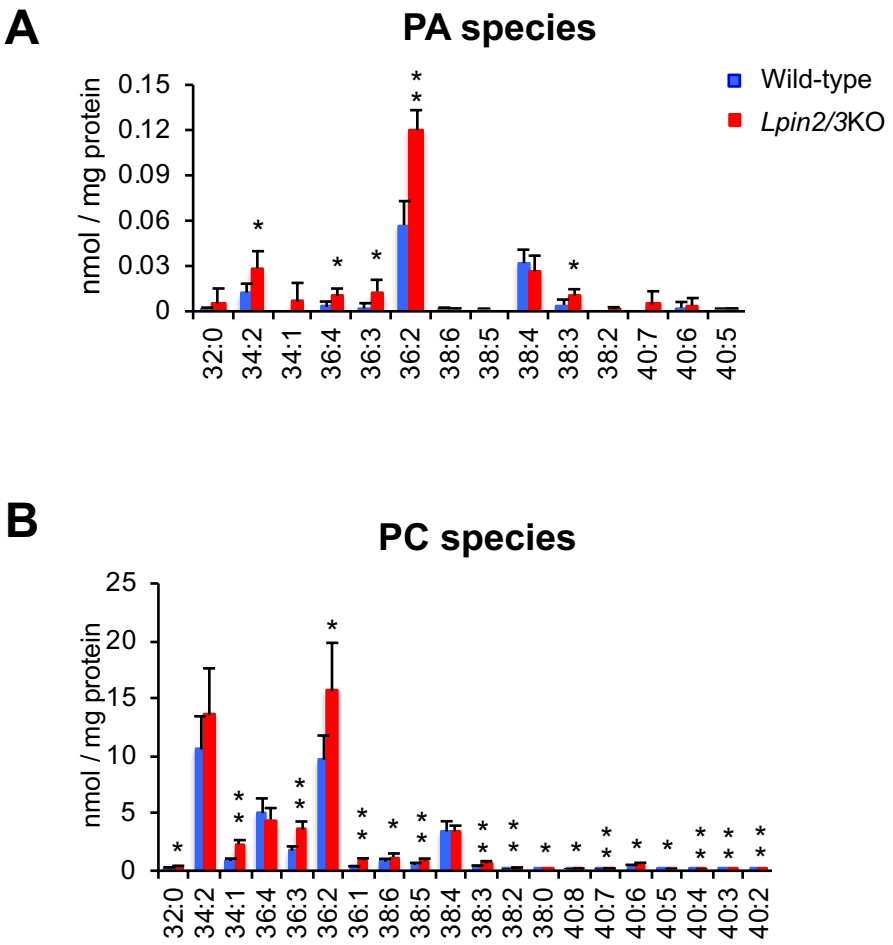


Fig. S6. Phospholipid species in *Lpin*2/3 KO mouse intestine.

(A) Phosphatiatidic acid (PA) species in proximal small intestine. Analysis was performed done by electrospray ionization mass spectrometry in mice after 6 days on high-fat diet. PA species are designated by their fatty acid components [total carbons:double bonds]. n = 4–6; . *, $p < 0.05$; **, $p < 0.01$.

(B) Phosphatidylcholine (PC) species in same samples and analysis as in S4A.

Fig. S7

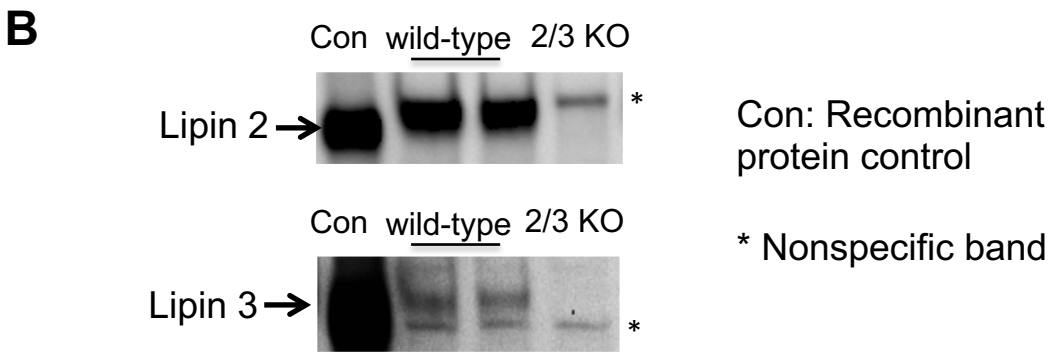


Fig. S7. Generation of *LPIN*2/3 KO cells by CRISPR-Cas9.

(A) Schematic of Cas9-sgRNA targeting sites in *LPIN2* and *LPIN3* and resulting protein sequence alterations in targeted *LPIN*2/3 KO HT-29 cell lines.

(B). Immunoblot analysis demonstrating lack of lipin 2 and lipin 3 protein in *LPIN*2/3 KO cell line (bands present in KO cell line are non-specific).

Fig. S8

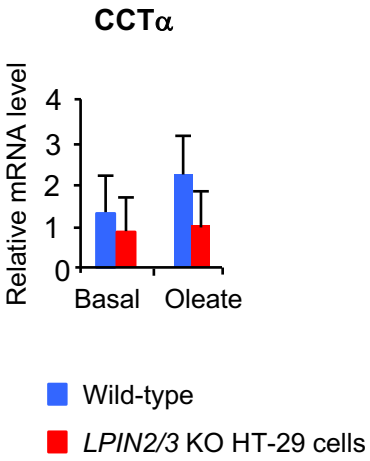


Fig. S8. CCTα mRNA levels in wild-type and *LPIN*2/3 KO HT-29 cells, quantified by qPCR and normalized to 36b4 and β2 microblobulin mRNA.