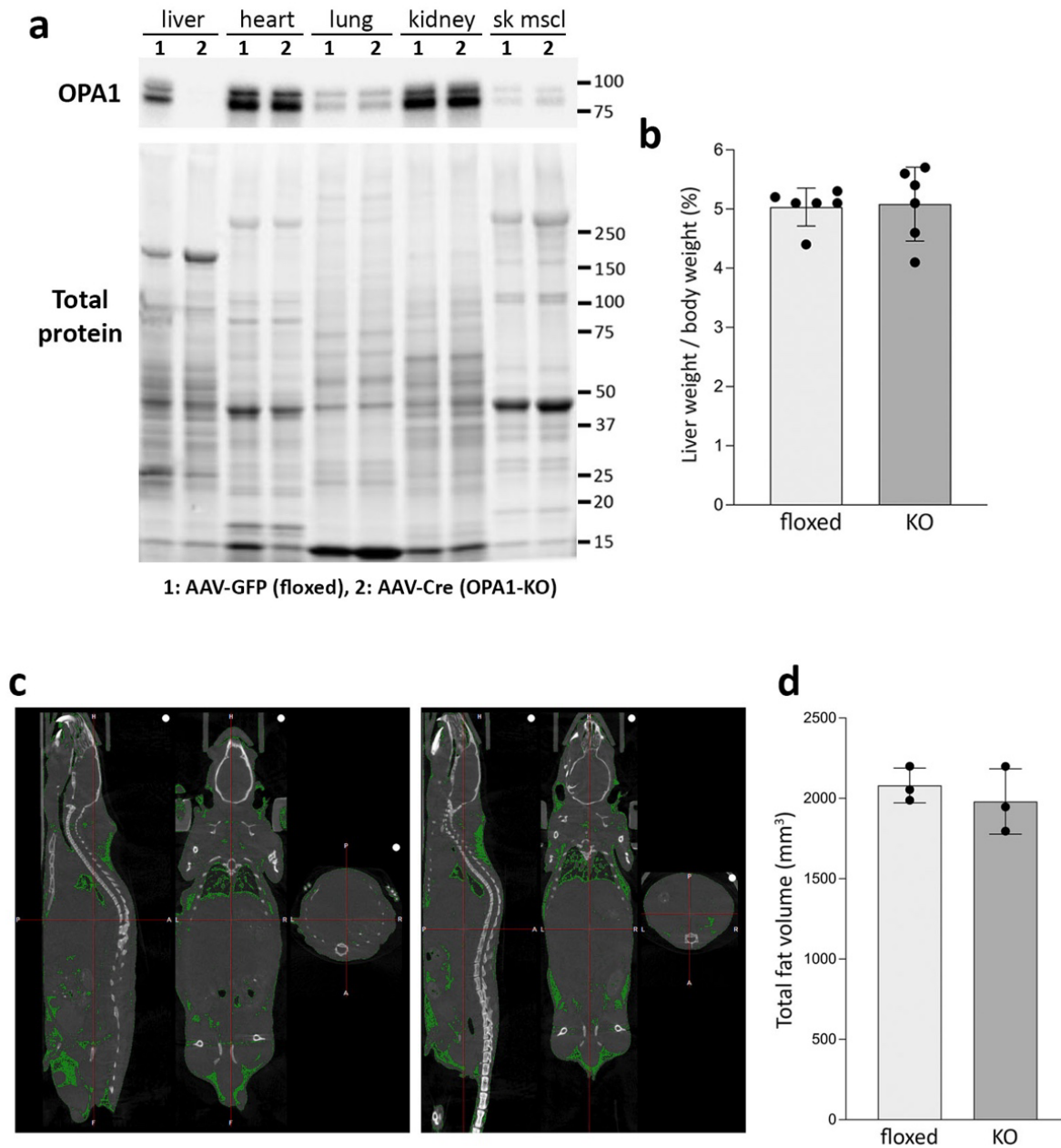
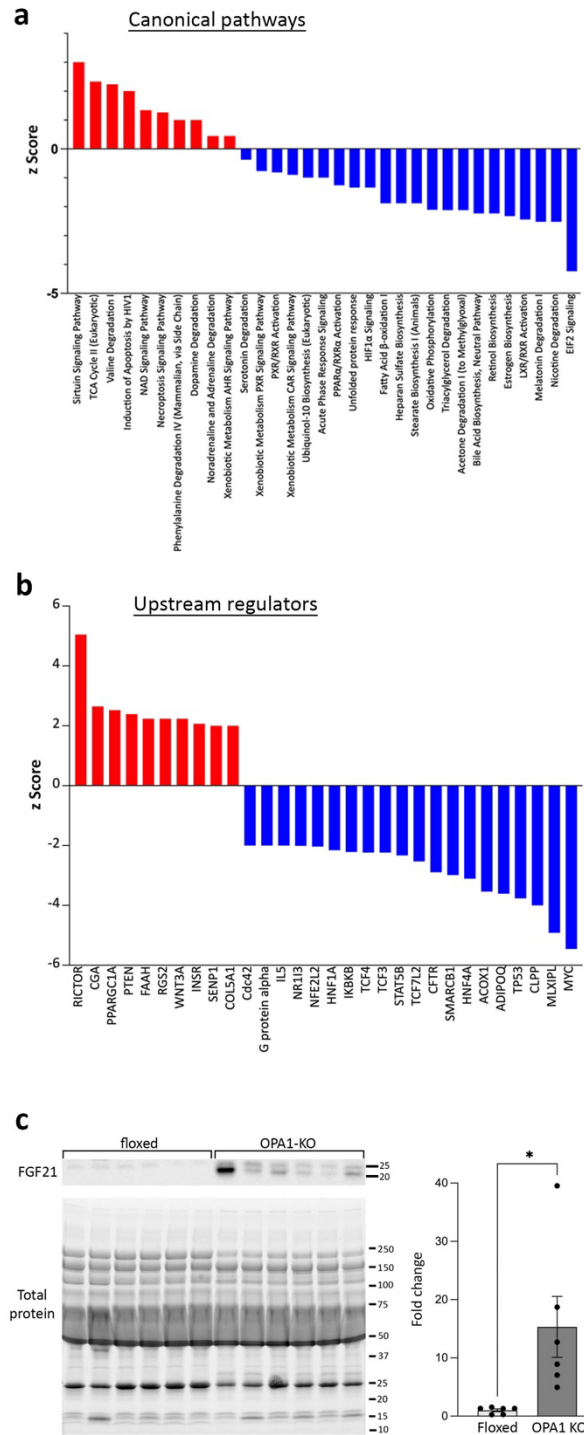


Suppl. Fig. S1



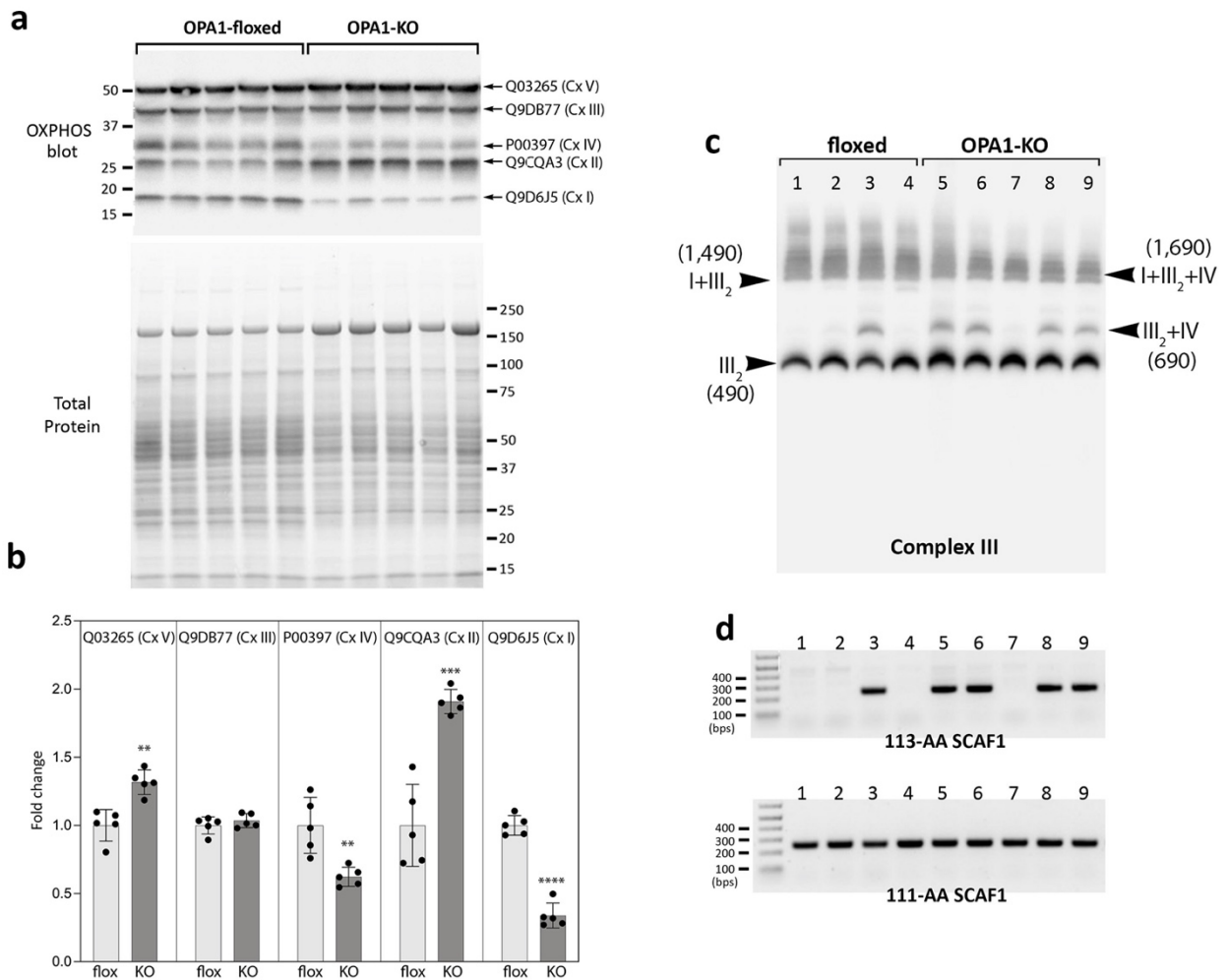
**Suppl. Fig. S1.** (a) AAV-TBG-Cre injection induces OPA1 KO specifically in the liver. (b) There is no difference in liver weight normalized to body weight. n=6 animals per group. Data are presented as mean values +/- SD. Unpaired t test. (c) Images of whole-body CT scan of mice. Green color denotes fat area. (d) Volume analyses of fat in floxed and OPA1-LKO mice. n=3 animals per group. Data are presented as mean values +/- SD. Unpaired t test. Source data are provided as a Source Data file.

## Suppl. Fig. S2



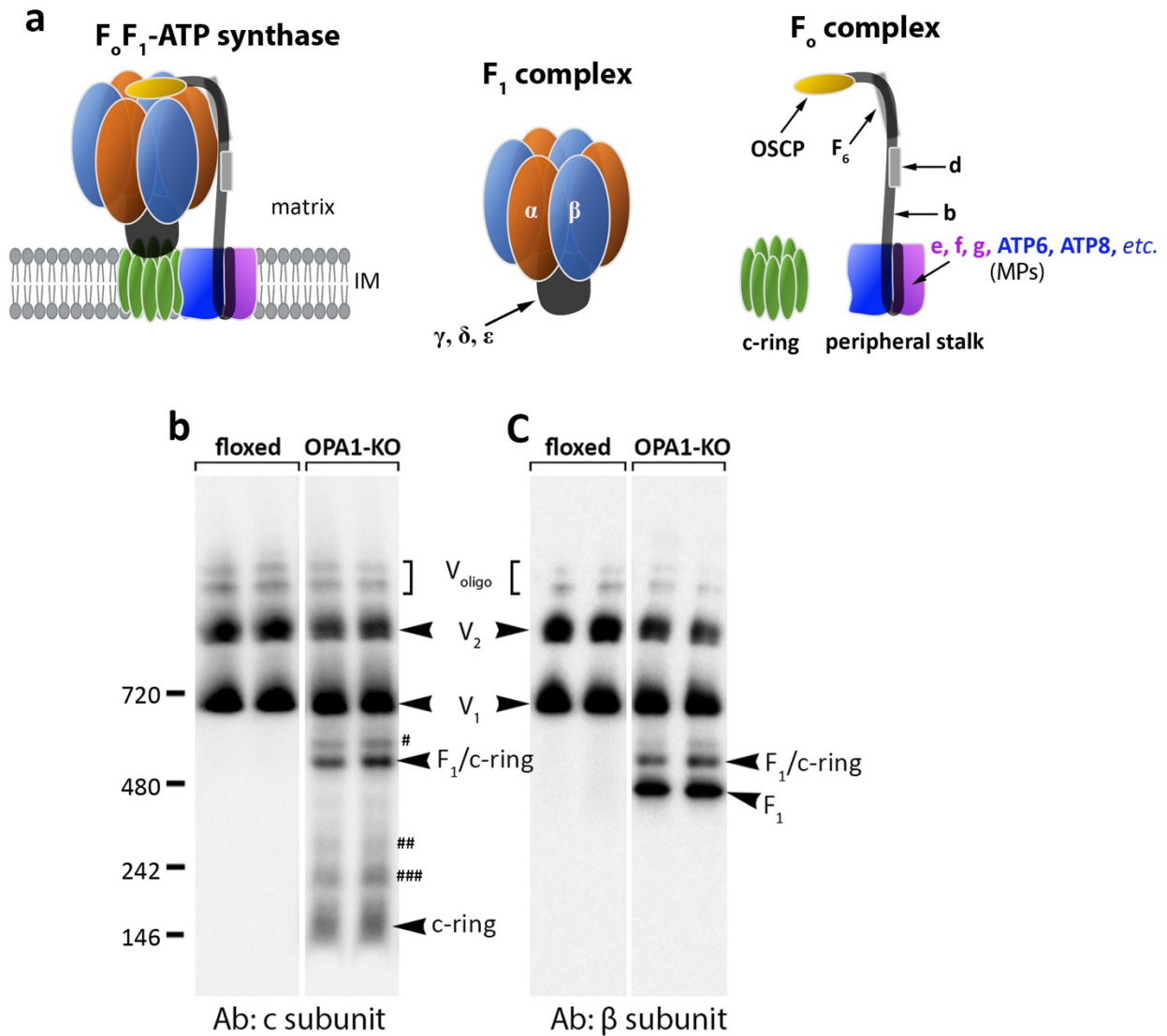
**Suppl. Fig. S2. (a, b)** IPA analyses of the proteomic data for canonical pathways and upstream regulators. **(c)** An increase of FGF21 in OPA1-LKO mouse serum. n=6 animals per group. Data are presented as mean values  $\pm$  SD. Unpaired t test. P=0.0211. Source data are provided as a Source Data file.

Suppl. Fig. S3



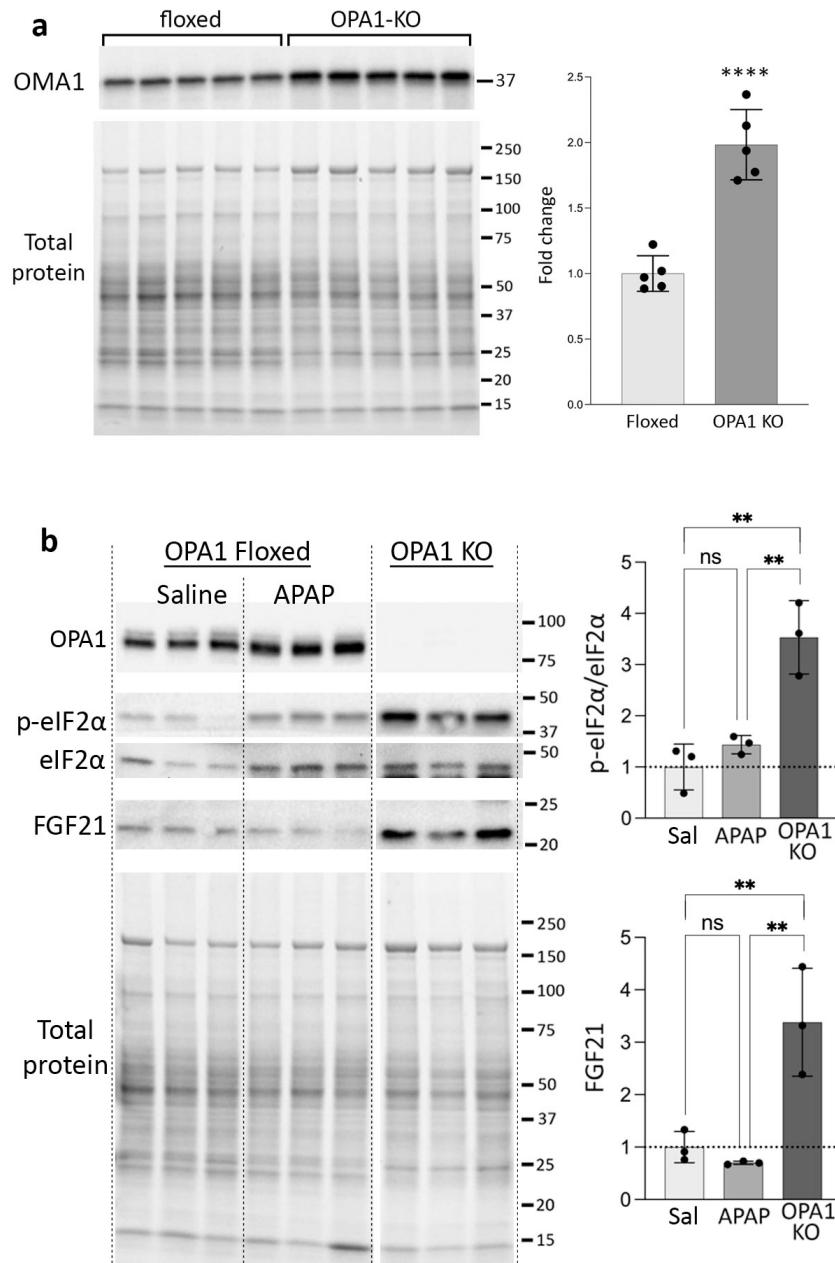
**Suppl. Fig. S3. (a)** Immunoblot of control and OPA1-KO liver lysates using the OXPHOS complex antibody cocktail. **(b)** Quantification of the immunoblot. n=5 animals per group. Data are presented as mean values +/- SD. Unpaired t test. Cx V: p=0.00131, Cx IV: p=0.0046, Cx II: p=0.0002, Cx I: p<0.0001. **(c)** BNGE for complex III (same blot shown in figure 5d). **(d)** PCR for two SCAF1 isoforms in different mice. Source data are provided as a Source Data file.

Suppl. Fig. S4



**Suppl. Fig. S4. (a)** The subunits of complex V (F<sub>1</sub>F<sub>0</sub>-ATP synthase). The membrane-extrinsic F<sub>1</sub> complex contains α, β, γ, δ, and ε subunits. The F<sub>0</sub> complex is membrane-intrinsic and is composed of c-ring and peripheral stalk (b, OSCP, d, and F<sub>6</sub>) with multiple membrane proteins (MPs: e, f, g, ATP6, ATP8, etc.). **(b, c)** Subcomplexes accumulated in OPA1 KO mitochondria detected by antibodies against c and β subunits. In addition to c-ring and F<sub>1</sub>/c-ring, multiple c subunit-containing sub-complexes (#, ##, and ###) are present in OPA1 KO mitochondria.

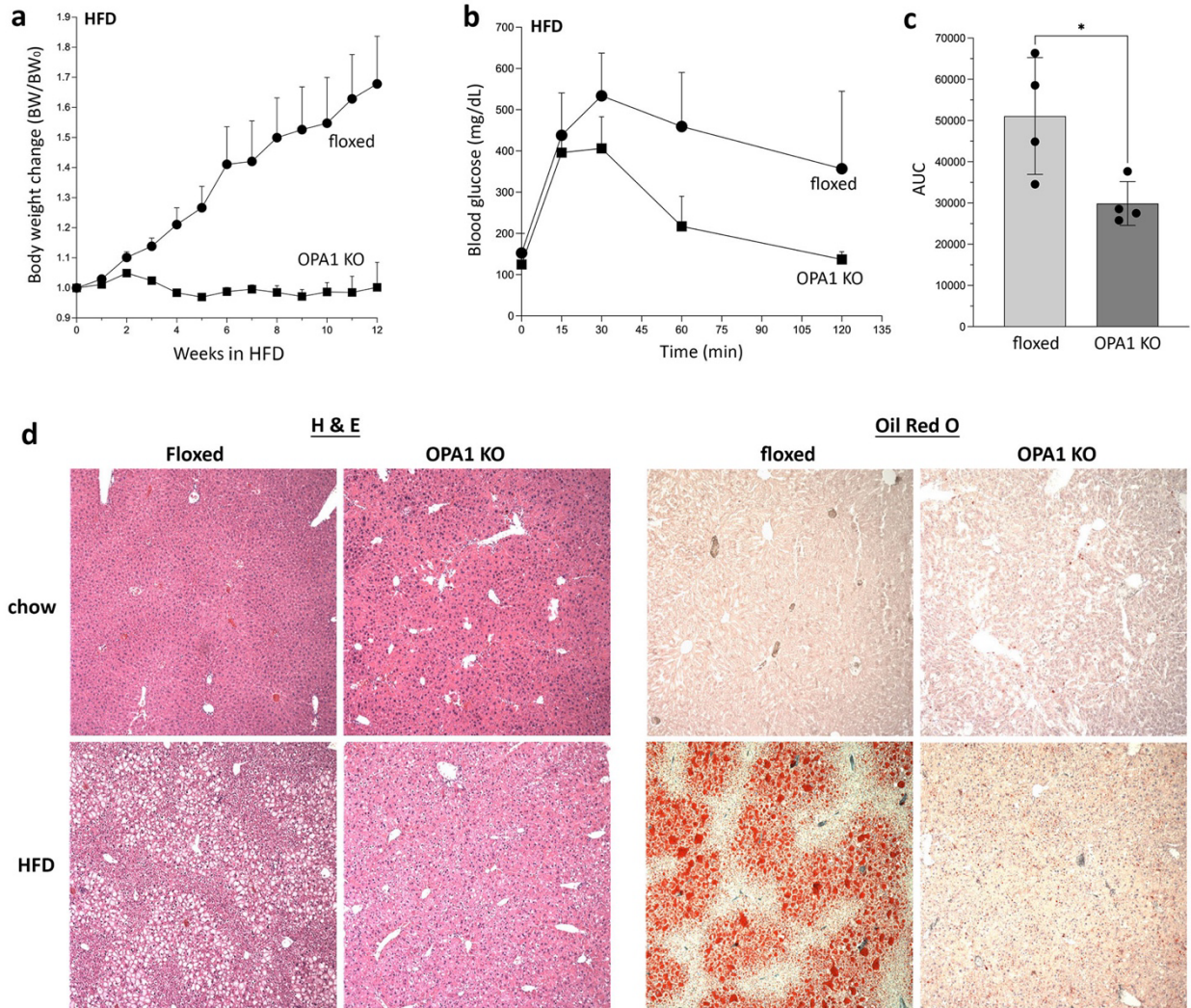
Suppl. Fig. S5



**Suppl. Fig. S5. (a)** OMA1 immunoblot of floxed and OPA1-KO livers. OPA1 KO significantly increased the OMA1 level. As OMA1 undergoes autocatalytic degradation upon activation, an increase in the OMA1 level indicates its inactivation. n=5 animals per group. Data are presented as mean values +/- SD. Unpaired t test.  $p < 0.0001$ . **(b)** APAP treatment does not induce the ISR. Quantification shows no significant change in ISR components in APAP treatment. OPA1-KO livers were included as positive control for ISR. n=3 animals per group. Data are presented as mean values +/- SD. One-way ANOVA with Turkeys multiple comparisons.  $p=0.0019$  and  $0.0051$  (p-eIF2 $\alpha$ );  $p=0.0078$  and  $0.0044$ . Source data are provided as a Source Data file.

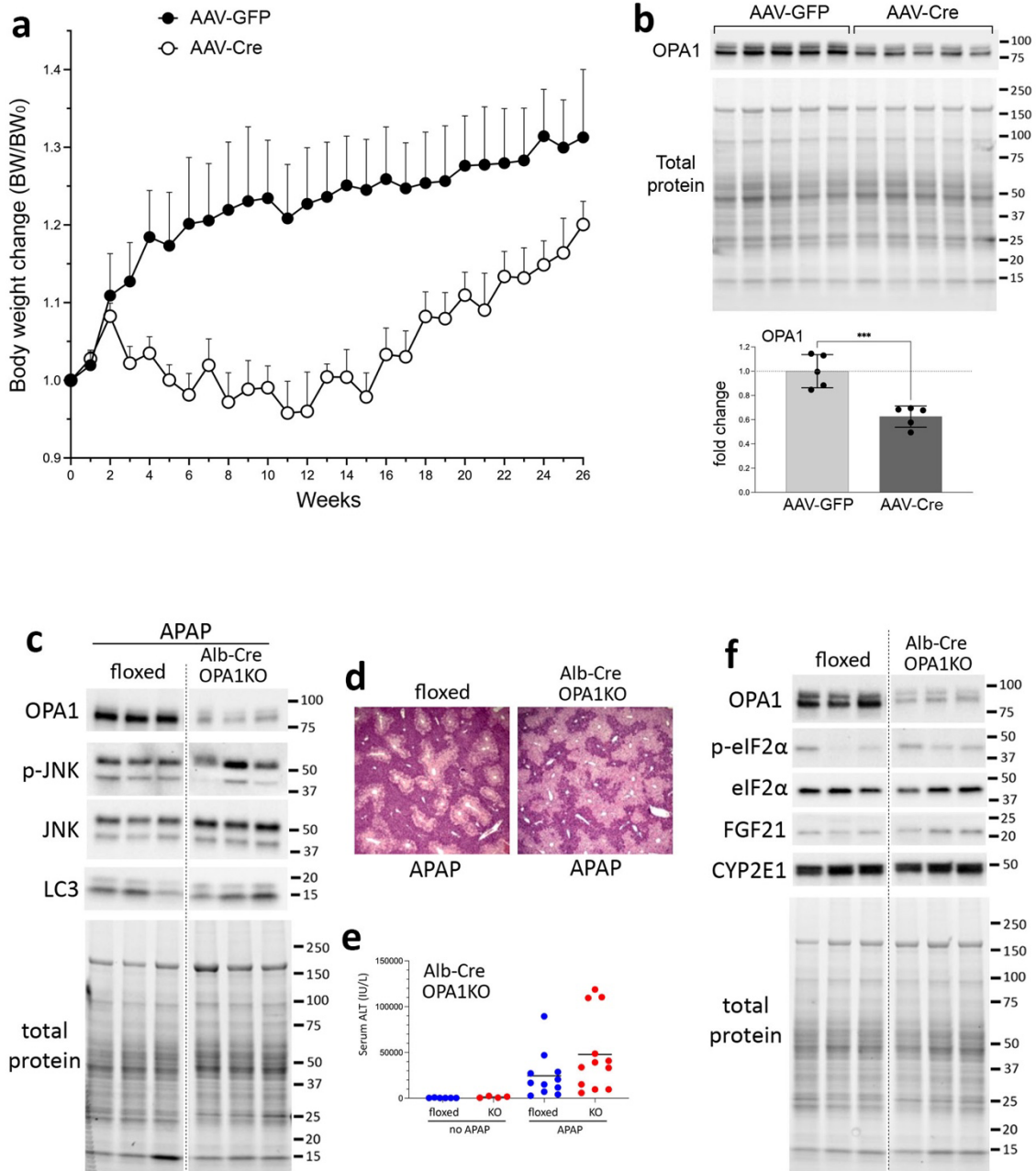


Suppl. Fig. S6



**Suppl. Fig. S6. Mitigation of HFD-induced obesity and hepatic steatosis by OPA1 LKO.** (a) Body weight change in high fat diet. (b, c) GTT of floxed and OPA1-KO mice after 12-week HFD. n=4 animals per group. Data are presented as mean values +/- SD. Unpaired t test. \*, p=0.0310. Source data are provided as a Source Data file. (d) Histology (H&E and Oil red O) showing marked reduction in liver steatosis by OPA1 LKO after 12-week HFD.

Suppl. Fig. S7



**Suppl. Fig. S7. (a)** Body weight change in an extended period after AAV-Cre administration. **(b)** OPA1 immunoblot at 24 weeks post-AAV-Cre. n=5 animals per group. Data are presented as mean values +/- SD. Unpaired t test. p=0.0009. **(c – f)** Alb-Cre-mediated OPA1 KO does not protect liver from APAP overdose. **(c)** There are no changes in JNK phosphorylation and LC3-II conversion in Alb-Cre-OPA1 KO compared with floxed controls. **(d)** H&E staining shows marked necrotic areas at 6 hours post APAP in both control and Alb-Cre-OPA1-KO livers. **(e)** Elevated ALT levels in Alb-Cre-OPA1-KO livers, similar to control. **(f)** There are no changes in eIF2 $\alpha$  phosphorylation and the levels of FGF21 and CYP2E1 in Alb-Cre-OPA1-KO livers, indicating no ISR induction. Source data are provided as a Source Data file.