Expanded View Figures

Figure EV1. MAPL SUMOylates ABCD3 and its absence alters ABCD3 complex assembly.

A Representative immunoblot of total lysate and streptavidin-beads pull-down fractions of HEK-293T-REX cells expressing Flag-MAPL-BirA or Flag-BirA (as control) incubated with biotin and probed for ABCD3, DRP1, MAPL and beta-actin.

B Quantification from three independent experiments of ABCD3 signals of the heavy membrane SIM-beads elution fraction (Fig 1C). *$P < 0.05$ in an unpaired two-tailed *t*-test.

C qRT-PCR investigating ABCD3 mRNA levels in livers of four different mice (2-month-old males).

D Representative immunoblots from whole-cell liver extracts probed for ABCD3, DRP1 and Mfn2 (Left panel). ABCD3, Drp1 and Mfn2 signals were quantified ($n = 3$ for each genotype, right panel).

E Representative immunoblots from whole-cell liver extracts probed for PEX14, ACOX1 and SCP2 (Left panel). PEX14, ACOX1 and SCP2 signals were quantified ($n = 3$ for each genotype, right panel).

Data are represented as mean ± SEM.

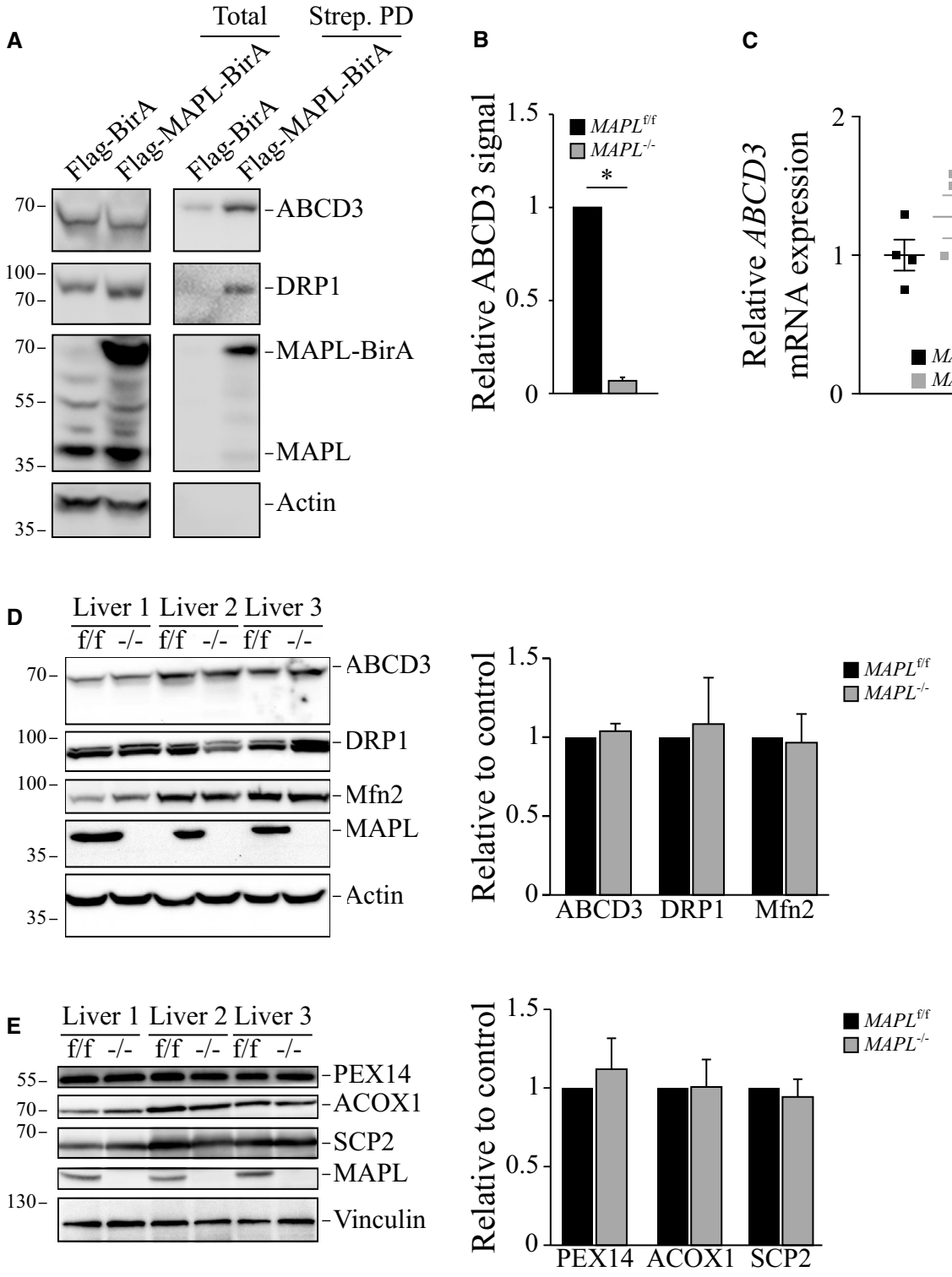

Figure EV1.

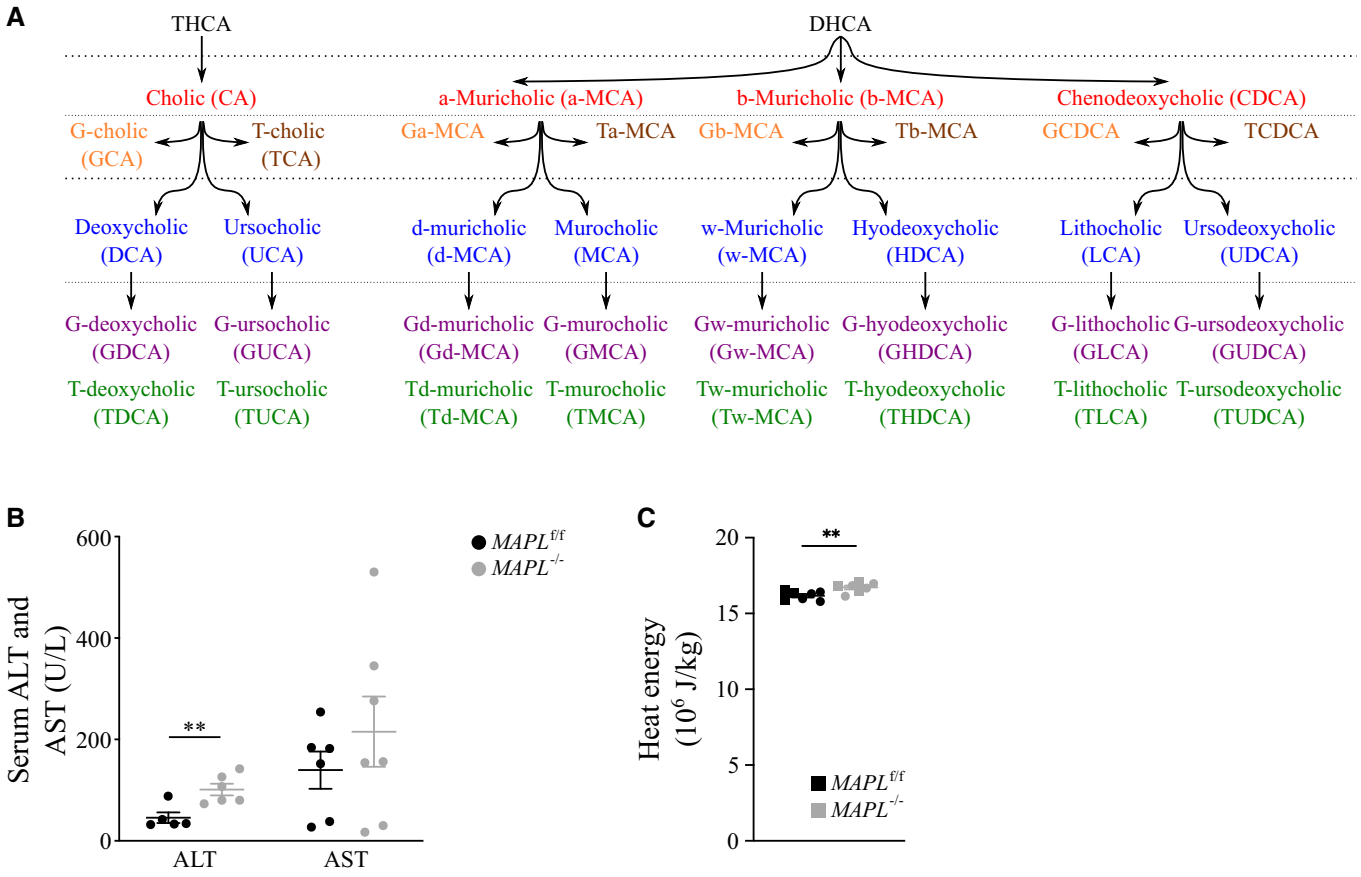

Figure EV2. Loss of MAPL alters bile acid synthesis.

A A model depicting the generation of the main murine bile acids from THCA and DHCA (tri- and dihydroxycholestanoic acids) (G: glyco-; T: Tauro-).

B Levels of alanine transaminase (ALT) and aspartate transaminase (AST), markers of liver damage, were quantified and plotted from serum collected from 6-month-old females (n = 5 and n = 6 for $MAPL^{f/f}$ and $MAPL^{-/-}$, respectively for ALT quantification and n = 6 and n = 7 for $MAPL^{f/f}$ and $MAPL^{-/-}$, respectively for AST quantification). **P < 0.01 in an unpaired two-tailed t-test.

C The amount of energy present in 3-month animal feces has been measured using an oxygen bomb calorimeter, as described in material and methods (n = 7 with four females and three males for each genotype). **P < 0.01 in an unpaired two-tailed t-test.

Data are represented as mean ± SEM. Squares represent males whereas circles represent females.

Figure EV3. Organellar gene expression in MAPL-deficient mice.

From liver RNAseq (N = 5 per sex and genotype). Volcano plots (left panels) and heat maps (right panels) of peroxisomal (top panels) and mitochondrial (bottom panels) gene expression in the liver of $MAPL^{-/-}$ mice.

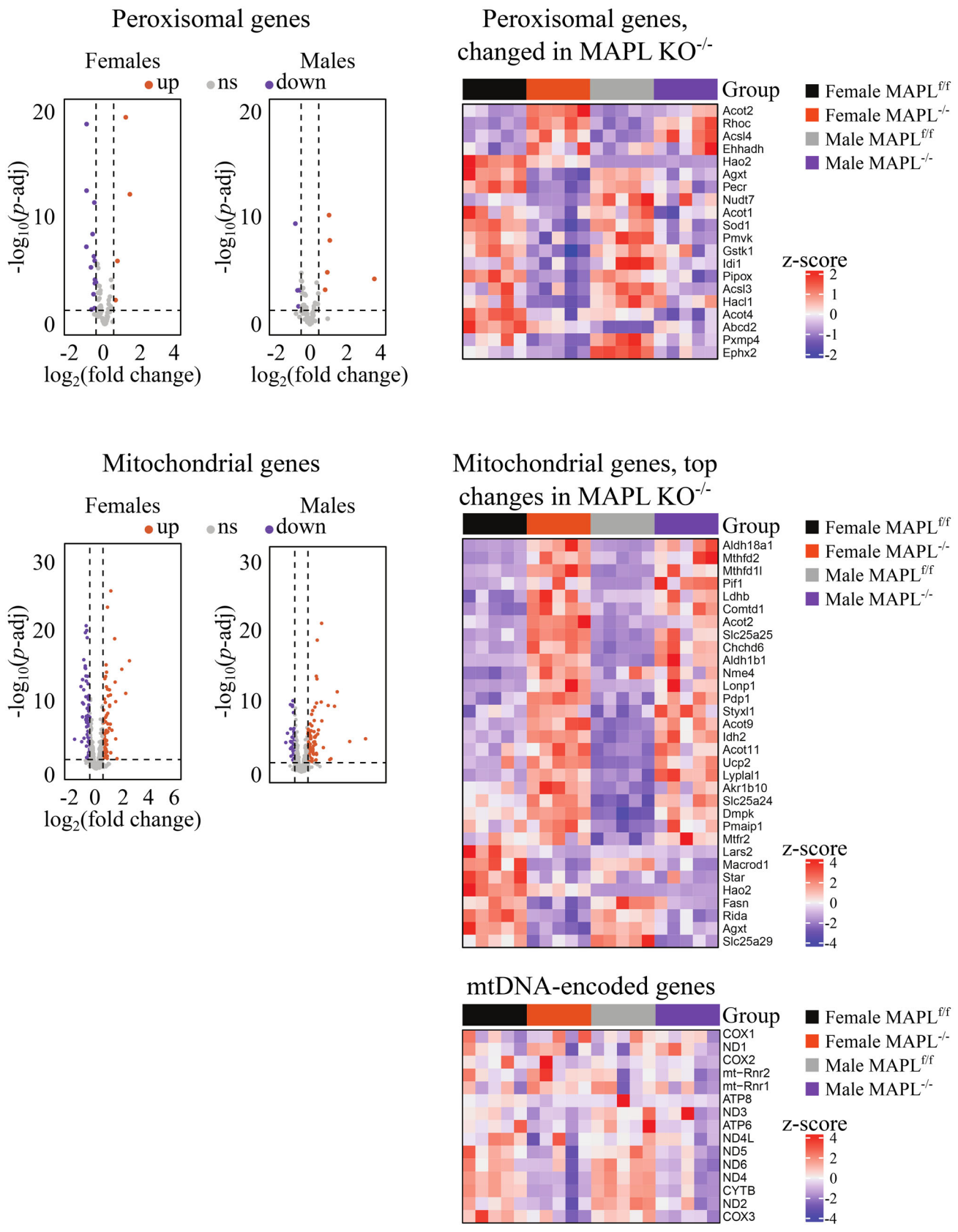
Peroxisomal genes
Females
Males
up
ns
down
-log10(p-adj)
log2(fold change)
Peroxisomal genes, changed in MAPL KO-/-
Group
Female MAPLf/f
Female MAPL-/-
Male MAPLf/f
Male MAPL-/-
Acot2
Rhoc
Acsl4
Ehhadh
Hao2
Agxt
Pecr
Nudt7
Acot1
Sod1
Pmvk
Gstk1
Idi1
Pipox
Acsl3
Hacl1
Acot4
Abcd2
Pxmp4
Ephx2
z-score
Mitochondrial genes
Mitochondrial genes, top changes in MAPL KO-/-
Aldh18a1
Mthfd2
Mthfd1l
Pif1
Ldhb
Comtd1
Acot2
Slc25a25
Chchd6
Aldh1b1
Nme4
Lonp1
Pdp1
Styxl1
Acot9
Idh2
Acot11
Ucp2
Lyplal1
Akr1b10
Slc25a24
Dmpk
Pmaip1
Mtfr2
Lars2
Macrod1
Star
Hao2
Fasn
Rida
Agxt
Slc25a29
mtDNA-encoded genes
COX1
ND1
COX2
mt-Rnr2
mt-Rnr1
ATP8
ND3
ATP6
ND4L
ND5
ND6
ND4
CYTB
ND2
COX3

Figure EV3.

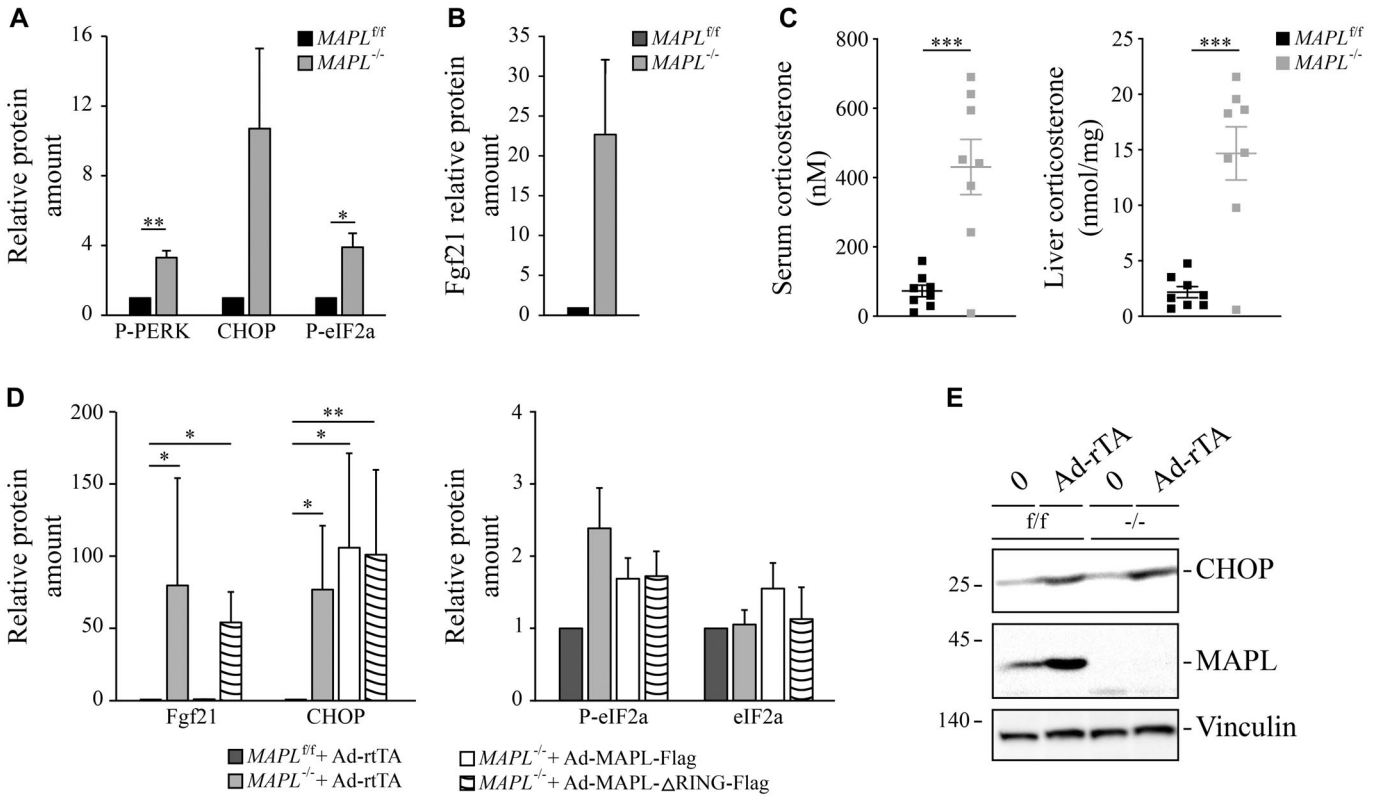

Figure EV4. Loss of MAPL leads to hepatic ER stress, eIF2α activation and Fgf21 expression.

A Quantification of phospho-PERK, CHOP and phospho-eIF2α signals presented in Fig 6A and B immunoblots (n = 3 different mouse for each genotype). *P < 0.05, **P < 0.01 in an unpaired two-tailed t-test.

B Quantification of Fgf21 signals presented in Fig 6E (n = 3 for each genotype).

C Circulating and liver corticosterone levels were quantified as described in materials and methods (n = 8 for each genotpye, 2-month-old males). ***P < 0.001 in an unpaired two-tailed t-test.

D Quantification of FGF21, CHOP, phospho-eIF2α and eIF2α signals presented in Fig 6J (n = 5, 5, 3 and 4 for MAPL$^{f/f}$ + rtTA, MAPL$^{-/-}$ + rtTA, MAPL$^{-/-}$ + MAPL-Flag and MAPL$^{-/-}$ + MAPL-ΔRING-Flag, respectively, 2–3 months old). *P < 0.05, **P < 0.01 in a Kruskal-Wallis test.

E Representative immunoblot of CHOP protein expression of livers isolated from MAPL$^{f/f}$ and MAPL$^{-/-}$ animals injected or not with the empty virus rtTA.

Data are represented as mean ± SEM. Squares represent males whereas circles represent females.

Figure EV5. MAPL$^{-/-}$ female mice are lean, resistant to weight gain and develop spontaneous hepatocellular carcinoma.

A Female mice fed with normal chow were weighed weekly; MAPL$^{f/f}$ (n = 7) and MAPL$^{-/-}$ (n = 10).

B MAPL$^{f/f}$ and MAPL$^{-/-}$ (n = 8 for each genotype) females were fed with normal chow for 5 months (dotted lines), or for 3 months followed by 2 months of 60% fat chow (solid lines, diet change indicated by HFD arrow).

C Body weight (g, left panel) and length (cm, right panel) of 7-month-old MAPL$^{f/f}$ (n = 4 for body weight and n = 3 for length) and MAPL$^{-/-}$ (n = 2) female mice. **P < 0.01 in an unpaired two-tailed t-test.

D Wet weight of organs including liver, kidney, gastrocnemius muscle, epididymal white fat (WAT) and interscapular brown fat (BAT) isolated from 7-month-old MAPL$^{f/f}$ (n = 4) or MAPL$^{-/-}$ (n = 2) female mice.

E White adipose tissue gene expression performed by qRT-PCR. N = 3, in triplicate, 2-month-old MAPL$^{fl/fl}$ and MAPL$^{-/-}$ male mice.

F Insulin tolerance test in MAPL$^{f/f}$ (n = 8), MAPL$^{-/-}$ (n = 8) female mice. Insulin (0.5 U/kg) was injected intraperitoneally after a 2 h fast and blood glucose was measured at indicated times. Repeated Measures 2way ANOVA analysis for a difference between genotypes P = 0.0135.

G Glucose tolerance test in MAPL$^{f/f}$ (n = 8), MAPL$^{-/-}$ (n = 8) female mice. Glucose (2 g/kg) was injected intraperitoneally after an overnight fast and blood glucose was measured at indicated times. Repeated Measures 2way ANOVA for a difference between genotypes P = 0.0903.

H Liver glycogen measured enzymatically in male MAPL$^{f/f}$ (n = 3), MAPL$^{-/-}$ (n = 3) mice. **P < 0.01 in an unpaired two-tailed t-test.

I Survival curve of MAPL$^{f/f}$ (n = 11) and MAPL$^{-/-}$ (n = 10) female mice.

J Cancer-free survival curve of MAPL$^{f/f}$ (n = 11) and MAPL$^{-/-}$ (n = 10) female mice.

Data are represented as mean ± SEM. Squares represent males whereas circles represent females.

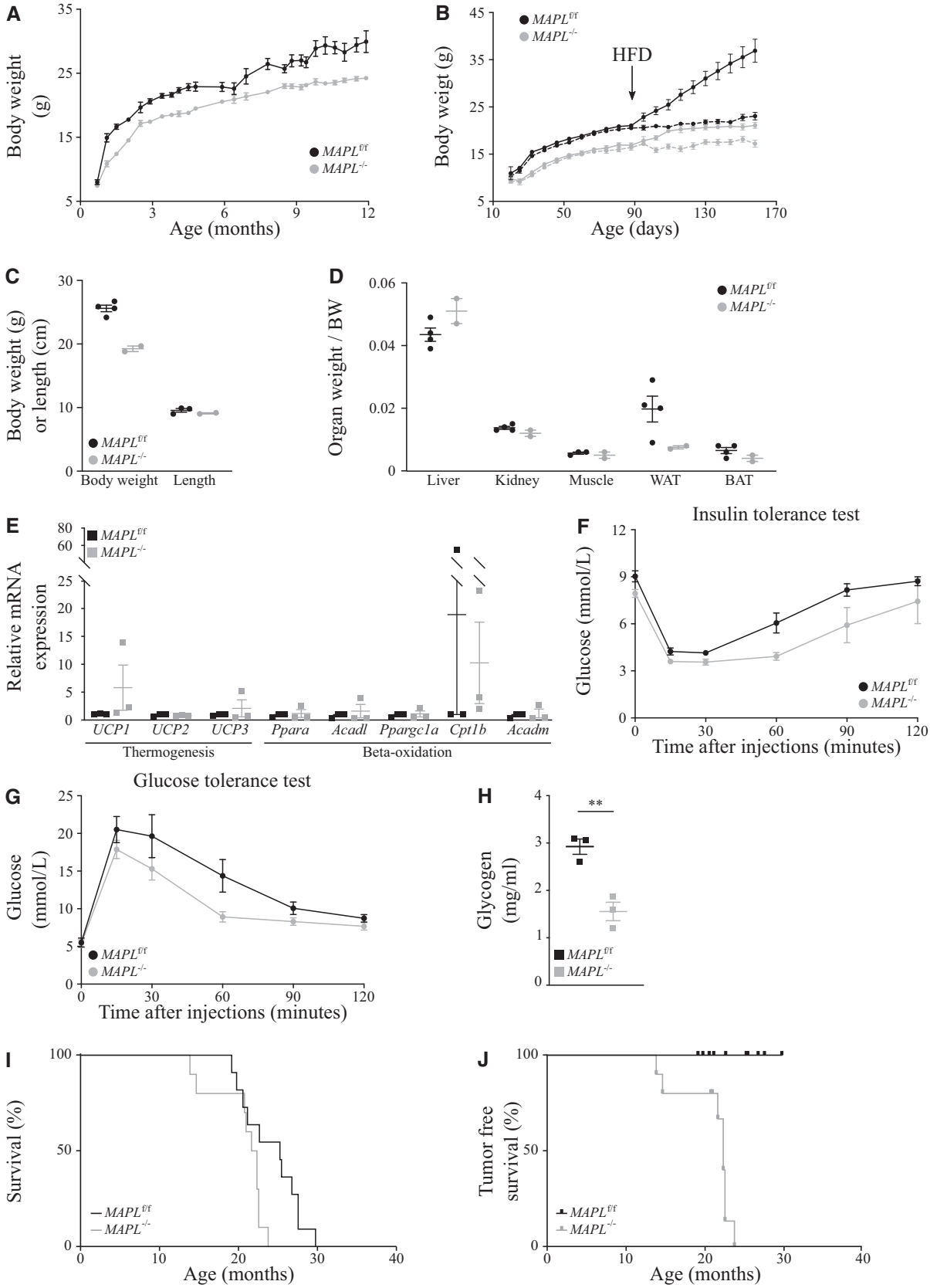
A
Body weight (g)
Age (months)
MAPL f/f
MAPL -/-
B
Body weigt (g)
HFD
Age (days)
C
Body weight (g) or length (cm)
Body weight
Length
D
Organ weight / BW
Liver
Kidney
Muscle
WAT
BAT
E
Relative mRNA expression
UCP1
UCP2
UCP3
Ppara
Acadl
Ppargc1a
Cpt1b
Acadm
Thermogenesis
Beta-oxidation
F
Insulin tolerance test
Glucose (mmol/L)
Time after injections (minutes)
G
Glucose tolerance test
Glucose (mmol/L)
Time after injections (minutes)
H
Glycogen (mg/ml)
**
I
Survival (%)
Age (months)
J
Tumor free survival (%)
Age (months)

Figure EV5.