Expanded View Figures

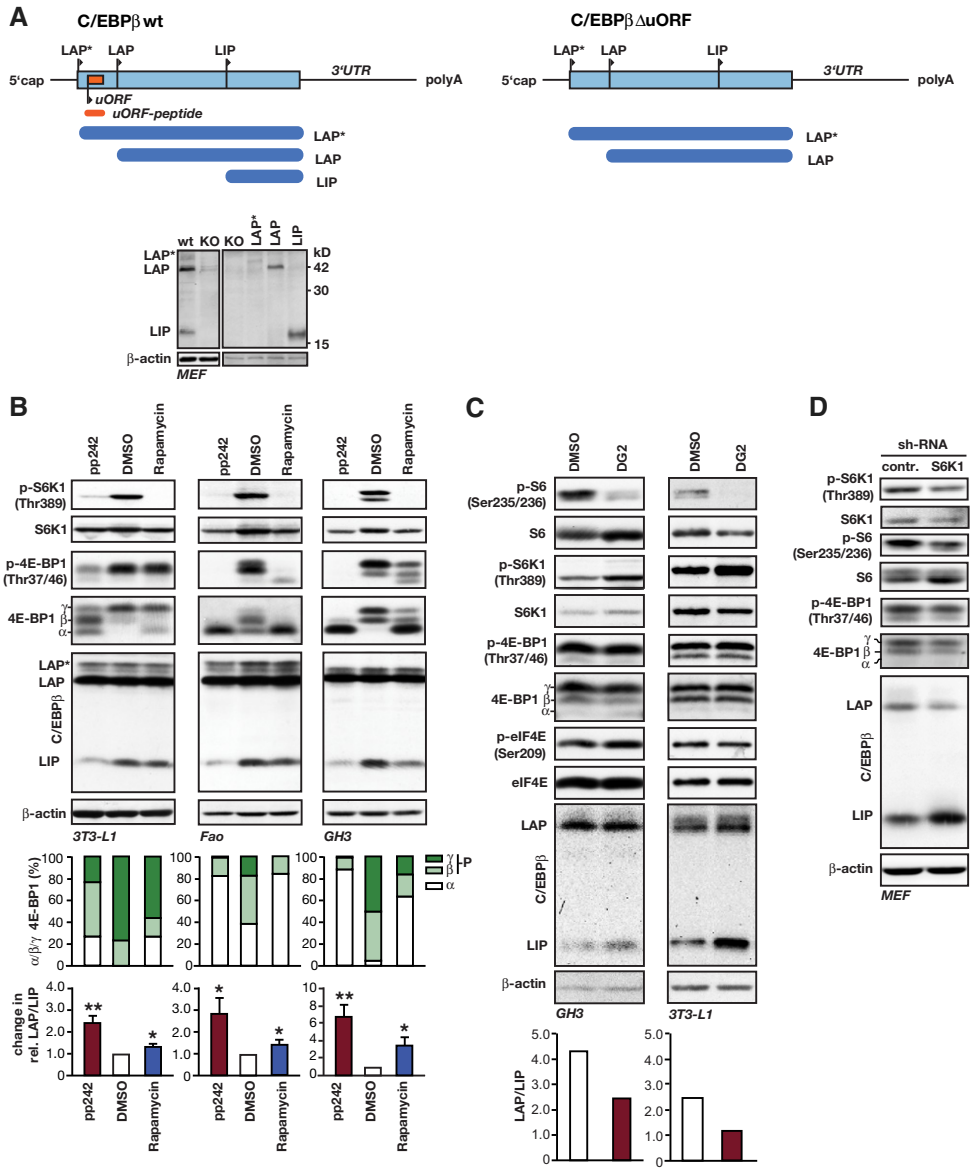

Figure EV1. Analyses of LAP/LIP C/EBPβ-isoform expression and the mTORC1 signalling pathway.

A Schematic view of C/EBPβ wt (left) and ΔuORF (right) mRNA structure and translated isoforms LAP*, LAP and LIP as indicated. The immunoblots at the left show wt MEFs and KO MEFs derived from C/EBPβ KO mice and at the right KO MEFs ectopically expressing empty vector control, LAP* (weakly expressed in MEFs), LAP or LIP. β-actin was used as a loading control.

B Immunoblots of extracts from 3T3-L1 mouse adipocytes (24-h treatment), Fao rat hepatoma cells (6-h treatment) and GH3 rat pituitary cells (24-h treatment) treated with the pan-mTOR inhibitor pp242 (1 μM) or the allosteric mTORC1 inhibitor rapamycin (1 μM) compared to solvent (DMSO) showing phosphorylation (p-) in relation to total levels of the indicated proteins. β-actin was used as a loading control. Upper bar graphs show quantification of percentages of 4E-BP1 α- (hypophosphorylated), β- and γ-bands (hyperphosphorylated) of the pan-4E-BP1 blot shown. The lower bar graphs show quantification of the relative changes in LAP/LIP-isoform ratio by pp242 or rapamycin compared to solvent (3T3-L1, $n = 4$; Fao, $n = 6$; GH3, $n = 3$). All values are mean ± SEM. *P*-values were determined with Student's *t*-test, $*P < 0.05$; $**P < 0.01$.

C Immunoblots from extracts of GH3 and 3T3-L1 cells treated with the S6K1 inhibitor DG2 (20 μM) or solvent for 24 h. Phosphorylation (p-) in relation to total protein levels of indicated proteins is shown. β-actin was used as a loading control. The bar graph shows quantification of the relative change in LAP/LIP-isoform ratio by DG2 compared to solvent ($n = 1$).

D Immunoblots from extracts of MEFs after retroviral transduction with control-sh or S6K1-sh expression vector showing phosphorylation (p-) in relation to total levels of the indicated proteins. β-actin was used as a loading control ($n = 1$).

Data information: Quantification of the LAP/LIP C/EBPβ-isoform ratios was done from X-ray films for (B) and by chemiluminescence digital imaging for (C).

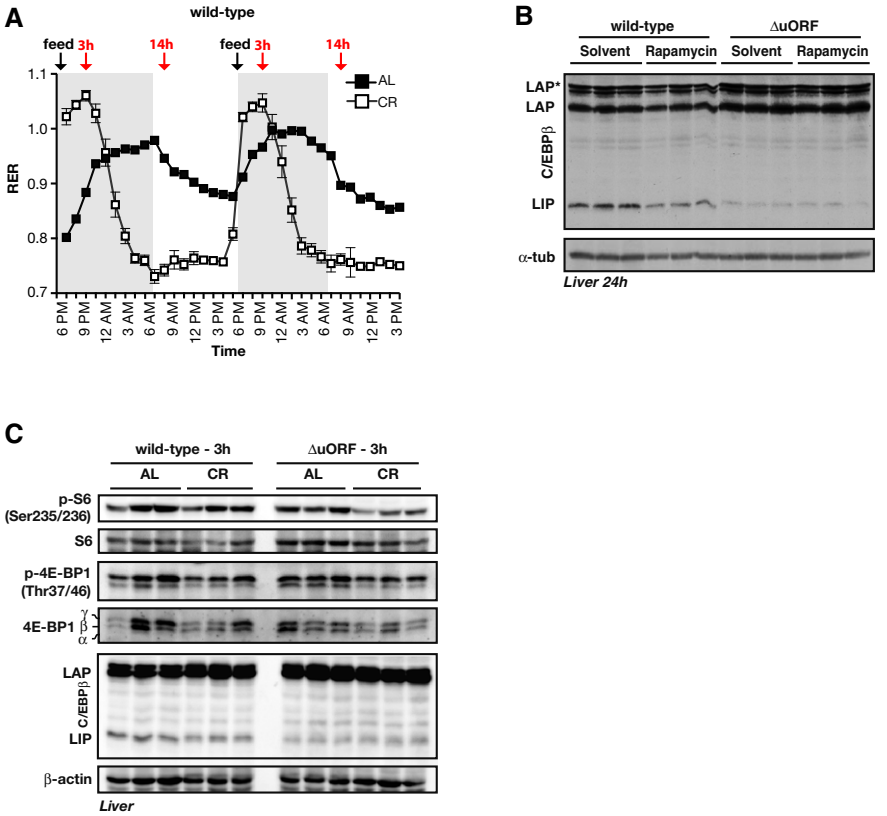

Figure EV2. C/EBPβ$^{\Delta uORF/BL6}$ mice data.

A Respiratory exchange ratio (RER) over 43 h of wt mice fed AL (black squares) or CR (open squares) for 4 weeks based on Oxymax measurements (n = 10). The dark phases are marked by grey boxes, and time of feeding is indicated as well as time points (3 and 14 h) used for immunoblotting shown in Fig 2C and E. All values are mean ± SEM.

B Whole immunoblot as shown separately in Fig 2A and D of extracts from livers of fed wt and C/EBPβ$^{\Delta uORF/BL6}$ mice 24 h after i.p. injection of rapamycin (8 μg/g body weight) or solvent. Phosphorylation (p-) in relation to total protein levels of indicated proteins is shown. α-tubulin was used as a loading control (n = 3).

C Whole immunoblot of extracts of livers from wt and C/EBPβ$^{\Delta uORF/BL6}$ mice (as shown separately in Fig 2C und E) either fed *ad libitum* (AL) or under caloric restriction (CR) for 4 weeks, sacrificed 3 h past-feeding (6 p.m. for CR). Phosphorylation (p-) in relation to total protein levels of indicated proteins is shown. β-actin was used as a loading control, n = 3.

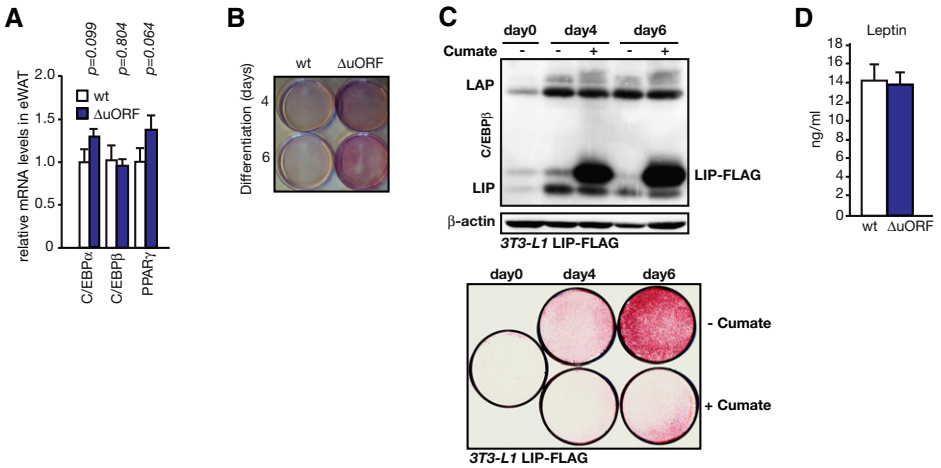

Figure EV3. Analyses of adipogenic function.

A mRNA expression levels in epididymal WAT from wt and C/EBPβ$^{\Delta uORF/BL6}$ mice of genes important for adipogenic differentiation analysed by qRT–PCR (wt, n = 5; C/EBPβ$^{\Delta uORF/BL6}$, n = 6).

B Lipid staining (Oil Red O) of primary MEFs isolated from wt or C/EBPβ$^{\Delta uORF/BL6}$ mice 4 and 6 days after adipogenic differentiation as indicated at the left side.

C Lipid staining (Oil Red O) of 3T3-L1 cells containing an ectopic cumate-inducible LIP-FLAG cassette either undifferentiated (day 0) or after 4 and 6 days of adipogenic differentiation with (+; LIP-FLAG induced) or without (–; not induced) cumate. Immunoblot shows induction of LIP-FLAG. β-actin was used as a loading control.

D Leptin levels in blood plasma (wt, n = 5; C/EBPβ$^{\Delta uORF/BL6}$, n = 6, measured in 8-month-old mice).

Data information: All values are mean ± SEM. P-values were determined with Student's t-test.

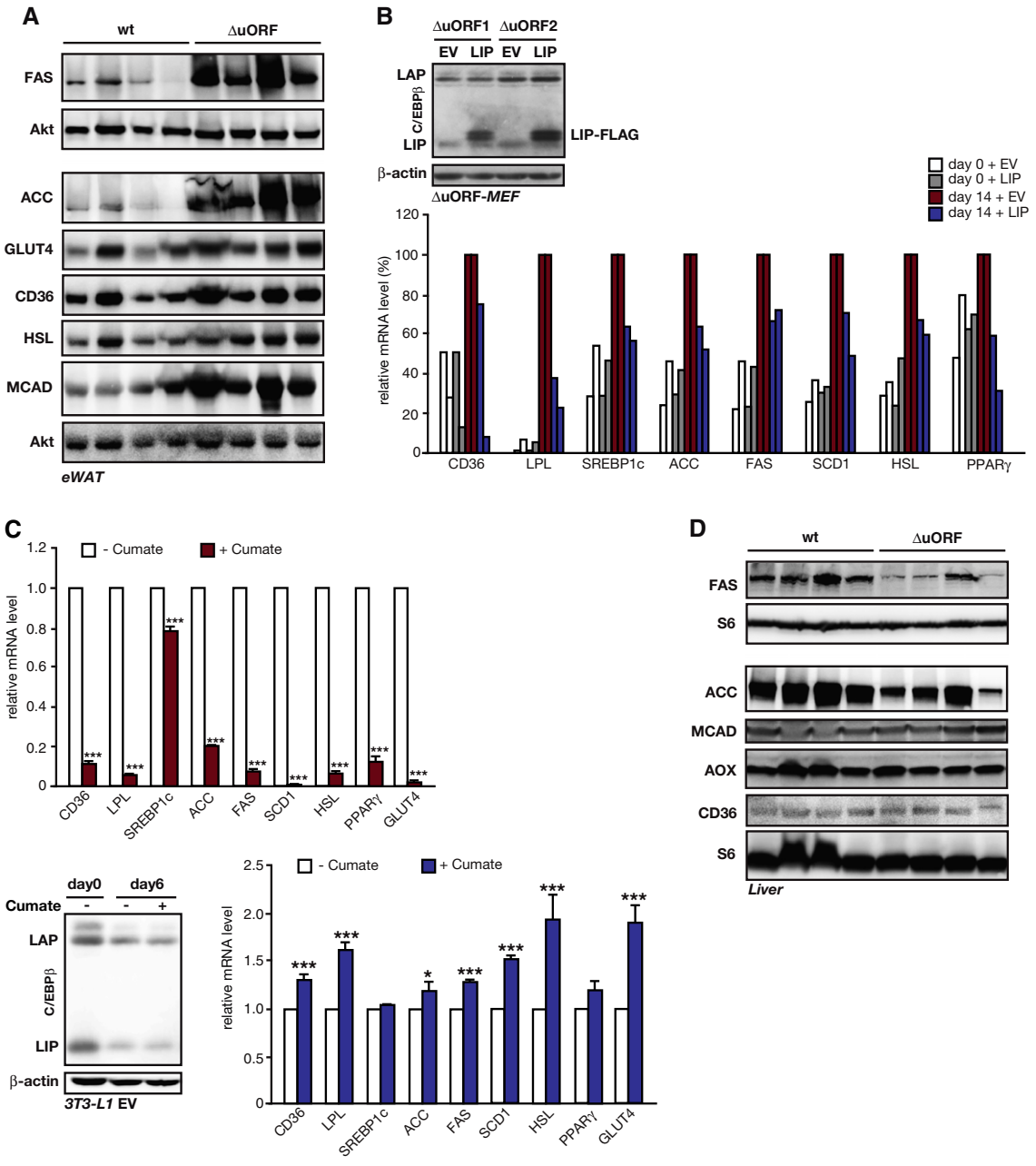

Figure EV4. Analyses of gene expression.

A Immunoblots of extracts from epididymal WAT of fed wt and C/EBPβ$^{\Delta uORF/BL6}$ mice with detection of the indicated proteins and Akt as a loading control (n = 4).

B Relative mRNA levels calculated from qRT–PCR data of indicated genes in C/EBPβ$^{\Delta uORF/BL6}$ MEFs 14 days after adipogenic differentiation compared to undifferentiated cells (day 0). Cells ectopically overexpressing LIP-FLAG (day 0/14 + LIP) were compared to empty vector control cells (day 0/14 + EV). mRNA levels are shown as percentage of day 14 controls. Immunoblot shows induction of LIP-FLAG, n = 2.

C Upper part: Relative mRNA levels calculated from qRT–PCR data of indicated genes in 3T3-L1 cells 6 days after adipogenic differentiation with cumate-inducible LIP-FLAG cultured without cumate (−; non-induced) or with cumate (+; LIP-FLAG induced). mRNA levels are shown relative to non-induced controls. Immunoblot for C/EBPβ expression in LIP-FLAG inducible cells upon differentiation without cumate (−) or with cumate (+) and β-actin as a loading control is shown in Fig EV3C. Lower part: Relative mRNA levels calculated from qRT–PCR data of indicated genes in 3T3-L1 cells 6 days after adipogenic differentiation with cumate-inducible empty vector (EV) cultured without cumate (−) or with cumate (+). mRNA levels of + cumate are shown relative to − cumate. Values are depicted as mean ± SD of three technical replicates, and corresponding P-values of the Student's t-test are depicted *$P < 0.05$; ***$P < 0.005$. Immunoblot shows C/EBPβ expression in empty vector (EV)-transfected cells upon differentiation without cumate (−) or with cumate (+). β-actin was used as a loading control.

D Immunoblots of extracts from livers of fed wt and C/EBPβ$^{\Delta uORF/BL6}$ mice with detection of the indicated proteins and S6 as a loading control (n = 4).

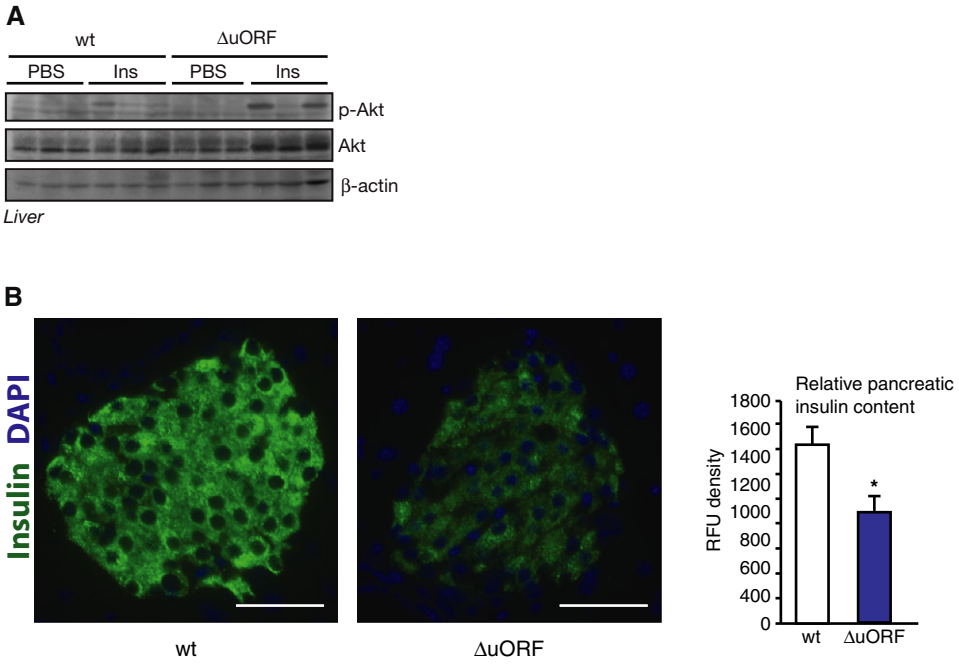

Figure EV5. Analysis of insulin signalling and pancreatic β-cells.

A Immunoblot showing Akt phosphorylation (p-Akt) (Thr308), Akt and β-actin protein levels in liver 10 min after i.v. administration of insulin (0.75 IU/kg) in 6 h-fasted wt and C/EBPβ$^{\Delta uORF/BL6}$ mice (n = 3).

B Fluorescent immunohistochemical analysis of the pancreas of wt or C/EBPβ$^{\Delta uORF/BL6}$ mice with insulin staining in green (anti-insulin antibody and Alexa Fluor 488-conjugated secondary antibody) and DAPI DNA staining in blue. Representative pancreatic islets are shown (scale bar corresponds to 50 μm). Relative quantification of insulin-specific fluorescence is shown at the right side (n = 6 mice, three islets each). Values are mean ± SEM, *P < 0.05.