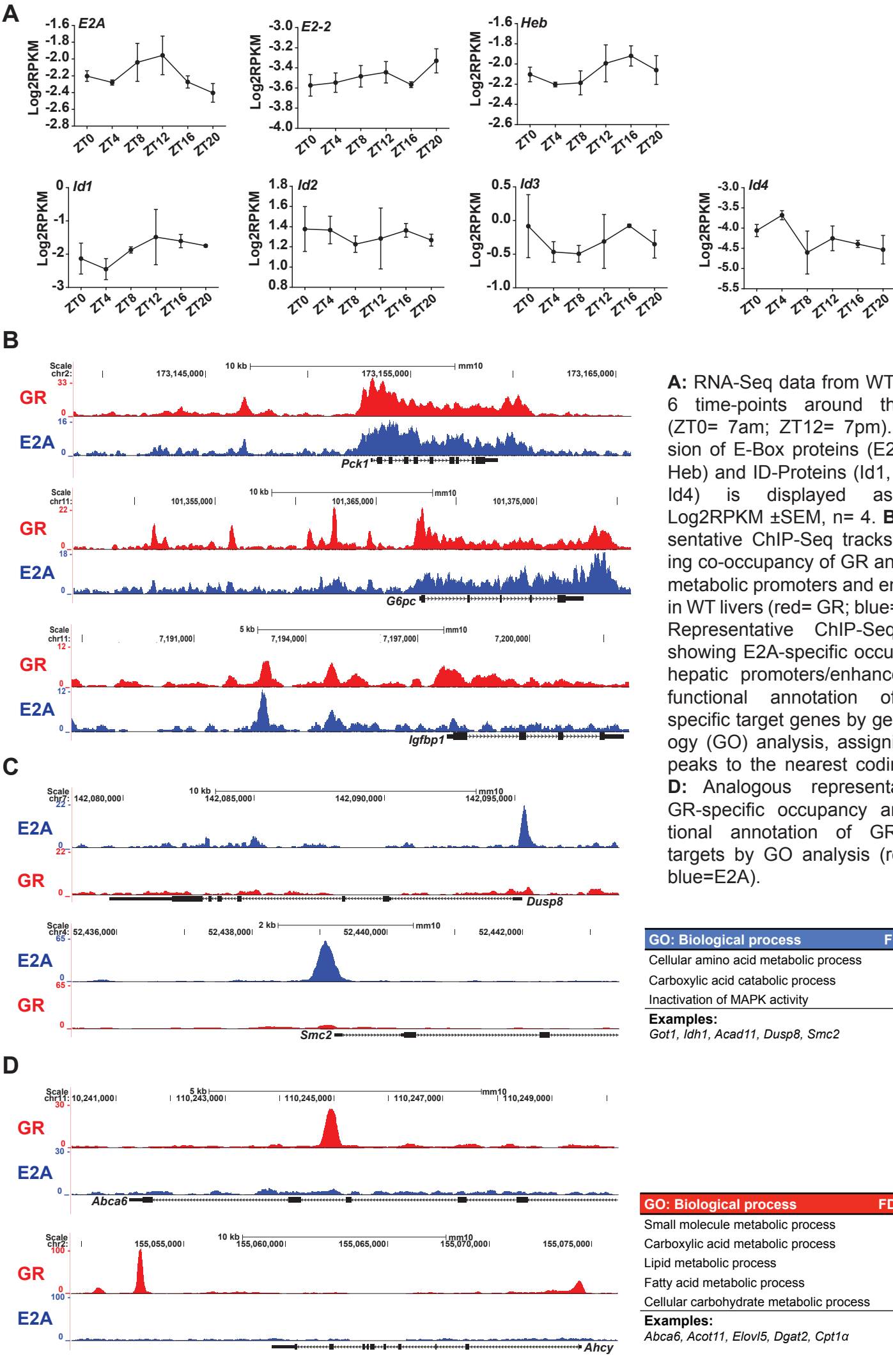
Supplementary Figures and Tables

Hemmer et al.

E47 mediates hepatic glucocorticoid action

Supplementary Figure 1: E2A expression and genomic binding in mouse liver.



A: RNA-Seq data from WT livers at 6 time-points around the clock (ZT0= 7am; ZT12= 7pm). Expression of E-Box proteins (E2A, E2-2, Heb) and ID-Proteins (Id1, Id2, Id3, Id4) is displayed as mean Log2RPKM ±SEM, n= 4. **B:** Representative ChIP-Seq tracks displaying co-occupancy of GR and E2A at metabolic promoters and enhancers in WT livers (red= GR; blue=E2A) **C:** Representative ChIP-Seq tracks showing E2A-specific occupancy at hepatic promoters/enhancers, and functional annotation of E2A-specific target genes by gene ontology (GO) analysis, assigning ChIP peaks to the nearest coding gene. **D:** Analogous representation of GR-specific occupancy and functional annotation of GR-specific targets by GO analysis (red= GR; blue=E2A).

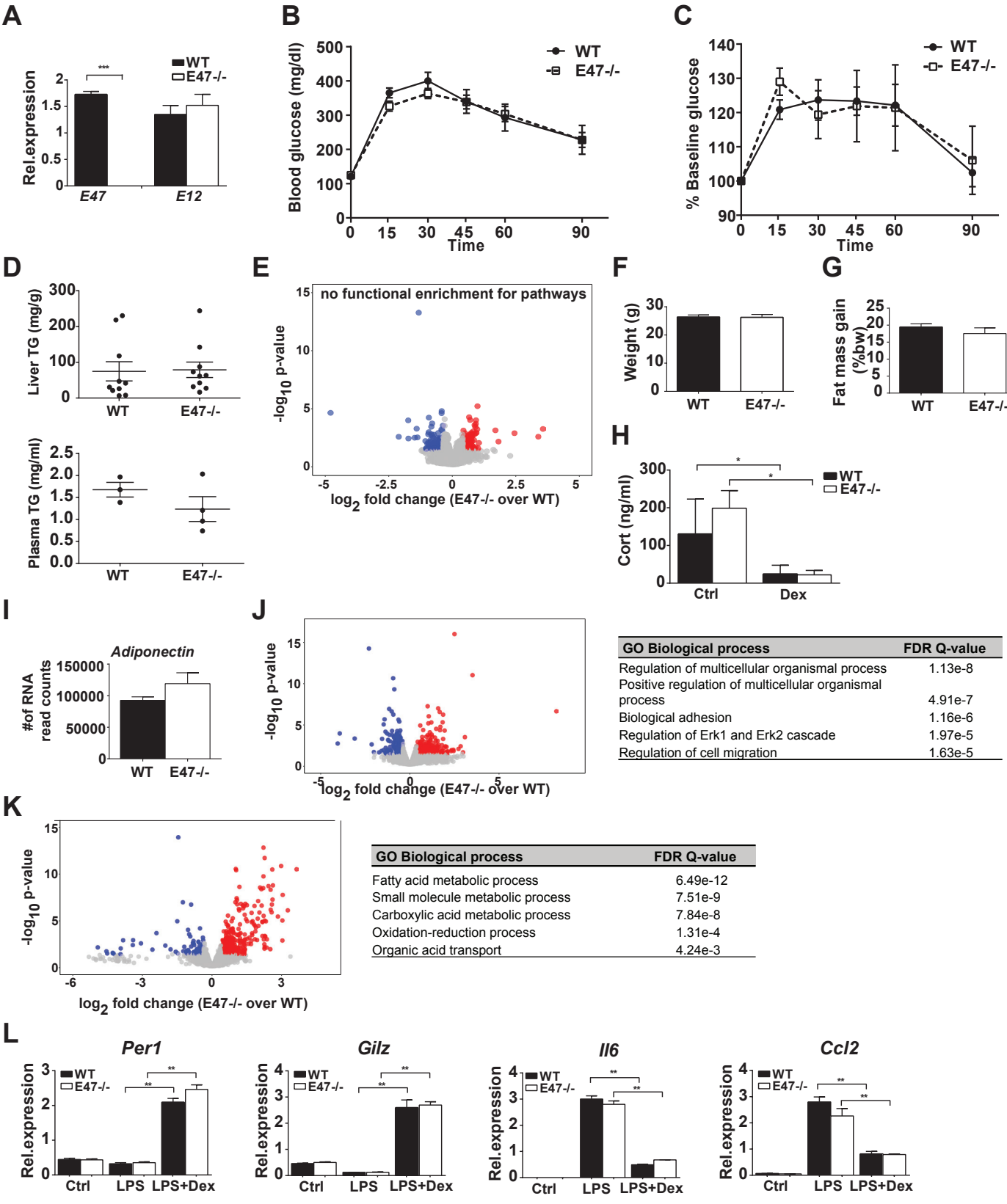
GO: Biological process	FDR Q-value
Cellular amino acid metabolic process	1.14e-2
Carboxylic acid catabolic process	2.23e-2
Inactivation of MAPK activity	2.14e-2

Examples:
Got1, Idh1, Acad11, Dusp8, Smc2

GO: Biological process	FDR Q-value
Small molecule metabolic process	1.52e-42
Carboxylic acid metabolic process	9.43e-37
Lipid metabolic process	6.99e-28
Fatty acid metabolic process	5.94e-20
Cellular carbohydrate metabolic process	9.28e-12

Examples:
Abca6, Acot11, Elovl5, Dgat2, Cpt1α

Supplementary Figure 2: Phenotypic characteristics and gene expression data from *E47-/-* mice.

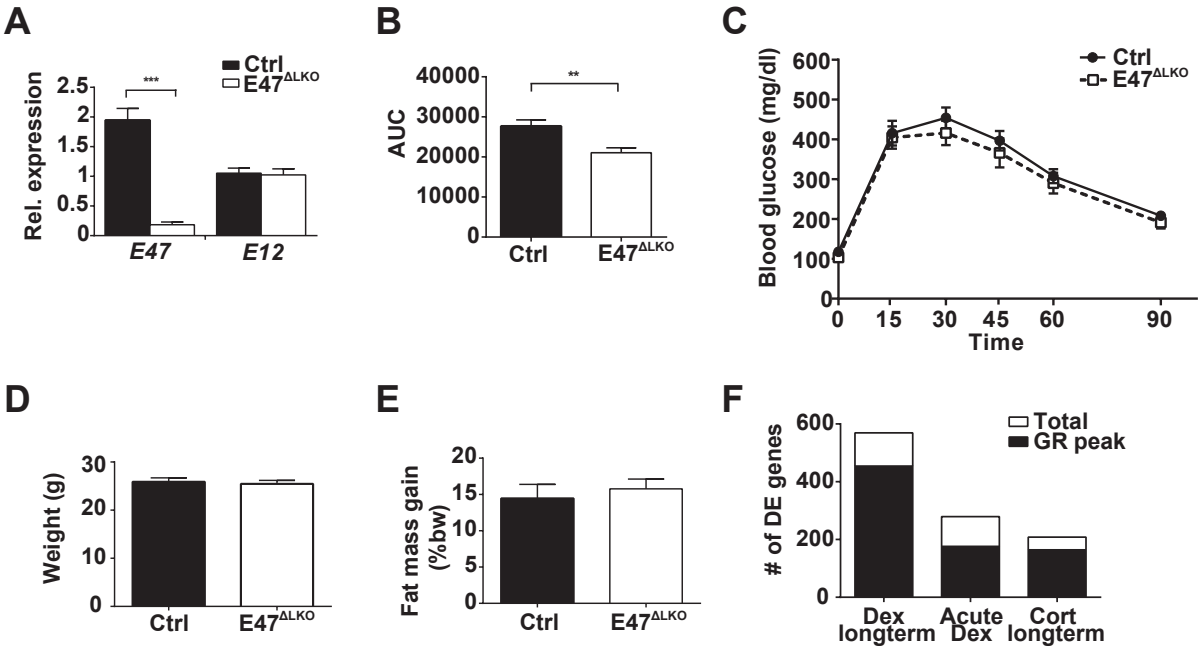


GO Biological process	FDR Q-value
Regulation of multicellular organismal process	1.13e-8
Positive regulation of multicellular organismal process	4.91e-7
Biological adhesion	1.16e-6
Regulation of Erk1 and Erk2 cascade	1.97e-5
Regulation of cell migration	1.63e-5

GO Biological process	FDR Q-value
Fatty acid metabolic process	6.49e-12
Small molecule metabolic process	7.51e-9
Carboxylic acid metabolic process	7.84e-8
Oxidation-reduction process	1.31e-4
Organic acid transport	4.24e-3

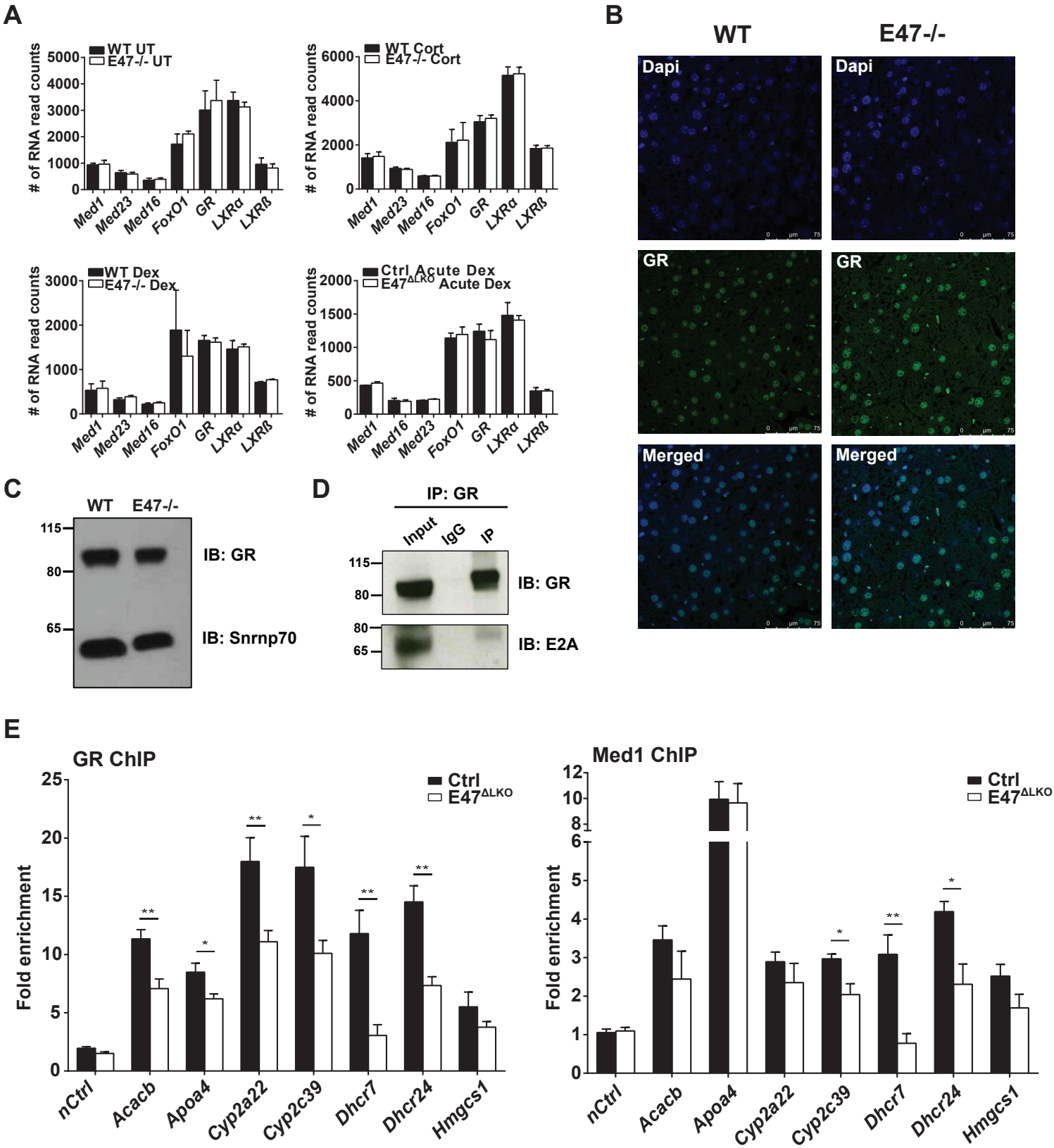
A: qRT-PCR of E47 and E12 expression in mouse livers after Dex treatment, normalized to U36b4. Data are expressed as mean ± SEM, (***) P<0.0001, Student's t-test, n=10 (WT) & 7 (E47-/-). **B:** i.p. GTT and **C:** i.p. PTT in untreated E47 -/- and WT mice. Data were analyzed by ANOVA and Bonferroni's multiple comparison test and are shown as mean ± SEM, n= 9 (WT) & 15 (E47-/-) for GTT; n= 6 (WT) & 7 (E47-/-) for PTT. **D:** Liver and plasma triglycerides in untreated mice. Data are mean ± SEM, n= 10 per genotype (liver); n= 3 (WT) & 4 (E47-/-) (plasma). **E:** Volcano plot and GO analysis of differentially expressed genes (blue= down-; red=up-regulated) in untreated mouse livers (FC 1.3, P<0.05, n= 5 (WT) & 6 (E47-/-)). For GO analysis a base mean cutoff >200 was used. **F:** Body weight of untreated E47-/- and WT mice. Data are mean ± SEM, n= 7 (WT) & 9 (E47-/-). **G:** Fat mass gain as percentage of body weight after Cort treatment. Data are mean ± SEM, n=8 (WT) & 11 (E47-/-). **H:** Plasma corticosterone of mice 6hrs after injection of 1mg/kg Dex or vehicle. Data are mean ± SEM, UT: n=7 per genotype; Dex-injected: n=6 (WT) & 5 (E47-/-). **I:** Average number of normalized NGS read counts for Adipoq in Dex-treated white adipose tissue. Data are mean ± STDEV, n=2 per genotype. **J:** Volcano plot and GO analysis of differentially expressed genes (blue= down-; red=up-regulated) in white adipose tissue (WAT) and skeletal muscle (SM) (**K**) of E47 -/- mice on Dex (FC 1.3, P<0.05); For GO analysis a base mean cutoff >200 was used; SM: n=2 (WT) & 3 (E47-/-); WAT: n= 2 per genotype. **L**: qRT-PCR in macrophages isolated from E47 -/- and WT mice and treated with LPS or LPS+Dex, normalized to U36b4; data are mean ± STDEV (**) P<0.01, (***) P<0.001, Student's t-test, n=2 per genotype.

Supplementary Figure 3: Measurements in *E47* liver specific mutant mice.



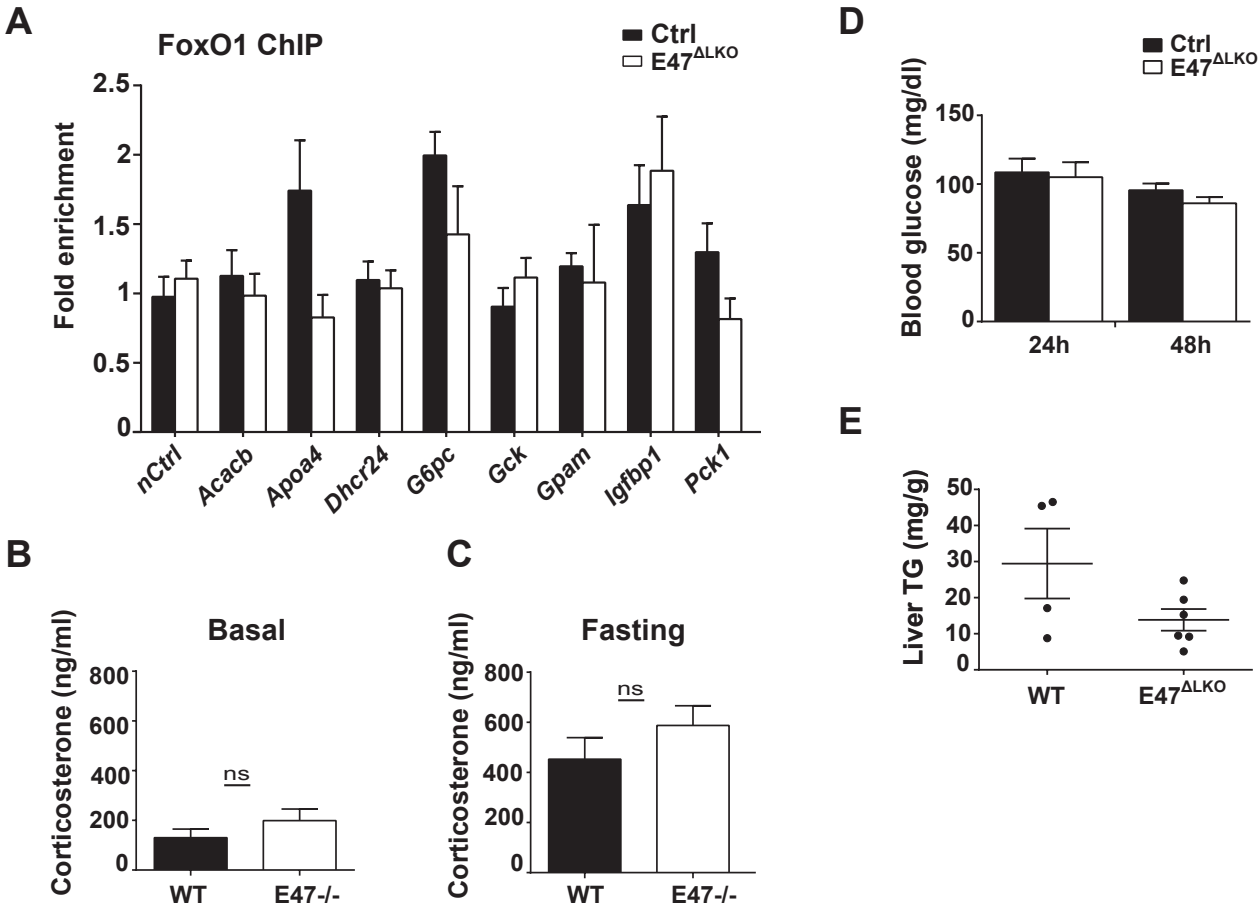
A: qRT-PCR of E47 and E12 expression in livers after Dex treatment, normalized to U36b4. Data are mean ± SEM, (***) P<0.0001, Student's t-test, n= 7 (Ctrl) & 5 (E47$^{\Delta LKO}$). **B:** Area under the curve (AUC) of GTT (Fig. **3A**). Student's t-test was performed on AUC, (**) P<0.01 **C:** i.p. GTT in untreated E47$^{\Delta LKO}$ and control mice. Data were analyzed by ANOVA and Bonferroni's multiple comparison test and are mean ± SEM, n=10 (WT) & 11 (E47$^{\Delta LKO}$). **D:** Body weight of untreated E47$^{\Delta LKO}$ and control mice. Data are mean ± SEM, n=7 per genotype. **E:** Fat mass gain as percentage of body weight on Cort. Data are mean ± SEM, n=8 per genotype. **F:** Number of genes differentially expressed in E47 mutant mice with a nearby GR ChIP peak (see Fig. **3G**).

Supplementary Figure 4: Expression levels and binding in mutant mice.



A: Number of normalized read counts for Med1, Med23, Med16, Foxo1, GR, Lxrα and Lxrβ from RNA-Seq data in livers either untreated (UT), Cort or Dex treated or Dex-injected (Fig. 2&3 and Suppl. Fig. 2). Data are mean ±STDEV. **B:** Immunostaining for GR in Dex-injected E47-/- and WT livers co-stained with DAPI, 63x magnification (75µm), confocal microscopy. **C:** Western blot of nuclear extracts from Dex-treated WT and E47-/- livers. Immunoblot (IB) for GR and Snrp70. **D:** Co-IP of GR and E2A in Dex-treated WT livers. Immunoblot (IB) for 30% input and IP against GR. **E:** ChIP-qPCR in Cort-treated E47$^{\Delta LKO}$ and control livers shows binding of GR & Med1 at metabolic promoters/enhancers. Data are shown as fold enrichment over IgG (n= 4 (Ctrl) and 6 (E47$^{\Delta LKO}$). Data are mean ±SEM, (*) P<0.05, (**) P<0.01, Student's t test.

Supplementary Figure 5: Data from fasted *E47* mutants.



A: ChIP-qPCR in fasted E47ΔLKO and control livers shows binding of Foxo1 at metabolic promoters/enhancers. Data are shown as fold enrichment over IgG, n= 6 (Ctrl) and 4 ($E47^{\Delta LKO}$). Data are mean ±SEM. **B:** Plasma corticosterone levels of untreated E47-/- mice and wildtype littermates, n=7 per genotype (Data taken from Supplementary Figure 2H). **C:** Plasma corticosterone levels of 48h-fasted E47-/- mice and wildtype littermates, n=8 (WT) & 5 (E47-/-). **D:** Blood glucose levels of $E47^{\Delta LKO}$ mice and control littermates measured at 24hrs and 48hrs of fasting. Data are mean ± SEM, n=4 (Ctrl) & 5-6 ($E47^{\Delta LKO}$). **E:** Liver triglycerides in 48h-fasted E47-/- mice and wildtype littermates. Data are mean ± SEM, n= 4 (Ctrl) & 6 ($E47^{\Delta LKO}$).

Supplementary Table 1a: Primers for ChIP qPCR

Abbreviation	Forward primer (5'-3')	Reverse primer (3'-5')
Acacb	CAGGCAGCGAGCATTTCCTA	TCTGATGCCCTTGTGCCTAC
Apoa4	TCACTGGGGTGGAAAGAGGA	CCTGAACAGAACTGAGGCCC
Cyp2a22	AAGGCCATCATGTACCTGGC	TGGCATGGATCTACAAAGGCT
Cyp2c39	GGGTTACTCAACGATGCTCAA	TTGTGATCAGGCATCACTGGC
Dhcr7	CCTGCGTAGCTTGGTTTCCTA	CAGAAGCTGGGCTATGACGG
Dhcr24	CTGGATGCCCTGTGAGTTCTA	ACAGGCATTCGCAAACATACT
G6pc	CAGGACGAAGGGAGAGAGC	AGTTCAGGCTGAGGACCTTG
Gck	GCTGTGGGTGAGGACTGTCT	TTTAGGAGCCACCTCTCAGACT
Gpam	ACACACAAGGAGGAGTGCAG	CACGGTTGCCCAATGAGTTA
Hmgcr	GGCCGCCAATAAGGAAGGAT	GGAGACCGTTCGTGACGTAG
Hmgcs1	TGGTCGGAGAACCTCTCACT	CGAGAACAAGCCTGCCAATG
Igfbp1	CAGCACTTTCCACCGTTGAC	GCAGCTCCAGAGTTAGGCAA
Pck1	CAGGGCTGTCCTCCCTTCTA	CTGTTGACCGAGGGTGTGTT
nCtrl	GCTGGCAGAATAGCATCCG	TGATGAAGCACTCGTTGAGGC

Supplementary Table 1b: Primers for mRNA

Abbreviation	Forward primer (5'-3')	Reverse primer (3'-5')
Acacb	CCTTTGGCAACAAGCAAGGTA	AGTCGTACACATAGGTGGTCC
Apoa4	CGTGGACCTGCAAGATCAGA	TCTGCATGCGCTGGATGTAT
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Cyp2a22	GTCACTCGCCTCTGCAAAAC	TGTACACTGGGCTTGGGAAC
Cyp2c39	GAGGAAGCATTCCAATGGTAGAA	TGTGAAGCGCCTAATCTCTTTC
Dhcr7	AGCTTCAGGCAGGCACTTAG	TGCTGGGATTTCGAAGCCAT
Dhcr24	CTGAAGACAAACCGGGAGGG	AAGATGGGGTTGTTGCCGAA
E12	TGCAGGATGAGCAGTTTGGT	GAGGCCTTTAAGGAGCTCGG
E47	TTATCCGACTTGAGGTGCAG	CTGGAGGAGAAGGACCTGAG
Gck	AACGACCCCTGCTTATCCTC	CTTCTGCATCCGGCTCATCA
Gpam	CTTCTAAGTCACCCACACCTGG	TTGCTTACTGGTCCTGTATCCT
Gilz	ACCACCTGATGTACGCTGTG	TCTGCTCCTTTAGGACCTCCA
G6pc	CGACTCGCTATCTCCAAGTG	GTTGAACCAGTCTCCGACC
Hmgcr	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC
Hmgcs1	GATCCCCTTTGGTGGCTGAAG	GAAAGAGCTGTGTGAAGGACAG
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Igfbp1	TCGTGACCACTGAGCAACTG	AGTTAGGAACTCGGGCATCG
Pck1	CTGCATAACGGTCTGGACTTC	CAGCAAACTCCCGTACTCC
U36b4	AGATTCGGGATATGCTGTTGGC	TCGGGTCCTAGACCAGTGTTC