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Supplemental Information

Estrogen Deficiency Promotes Hepatic Steatosis via a Glucocorticoid Receptor-Dependent Mechanism in Mice

Matthew A. Quinn, Xiaojiang Xu, Melania Ronfani, and John A. Cidlowski

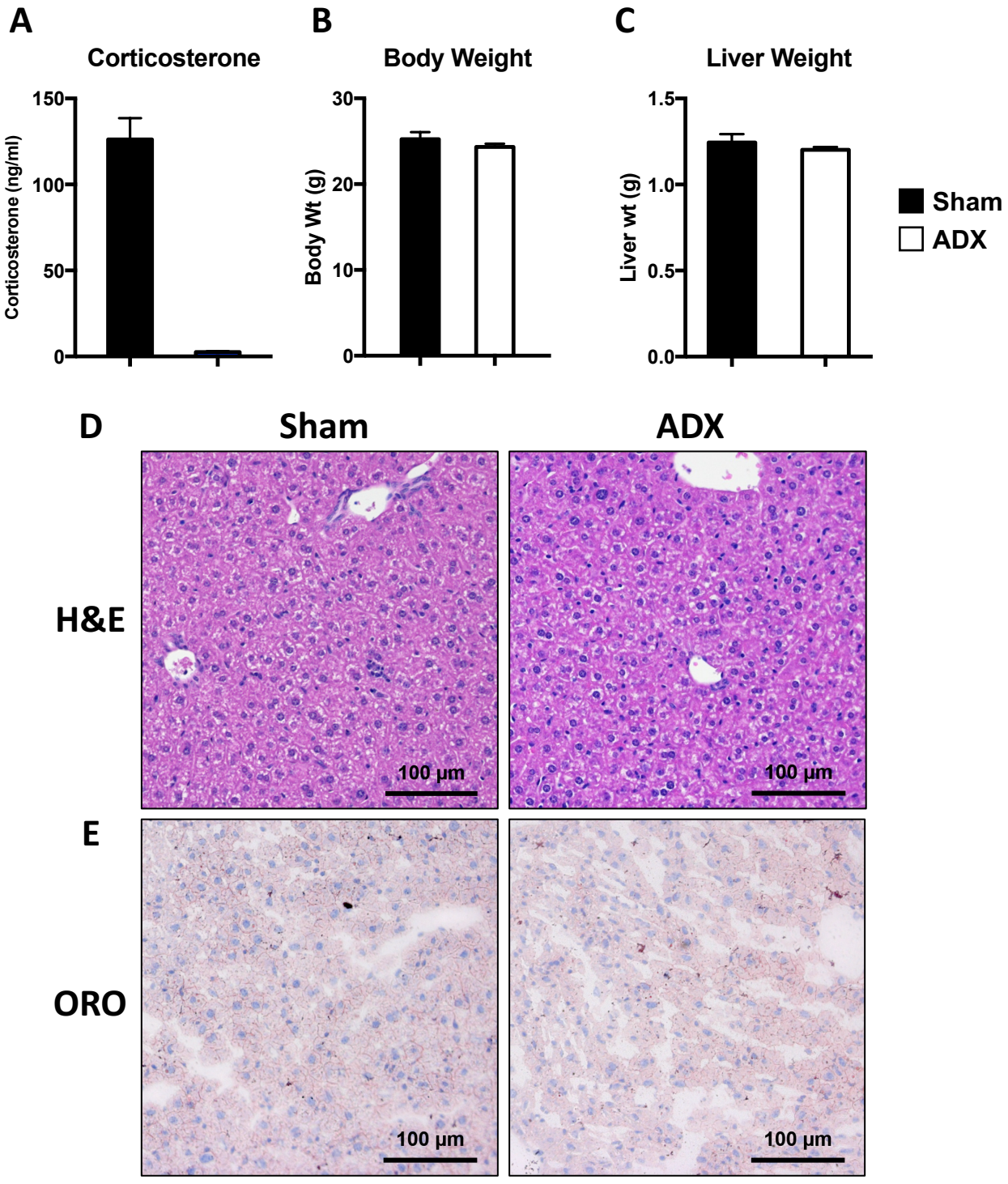

Figure S1: Adrenalectomy does not alter hepatic lipid composition. **Related to Figure 1. (A)** Circulating corticosterone levels in sham-operated and adrenalectomized mice. **(B)** Body weights and liver weights **(C)** of Sham and adrenalectomized mice. **(D)** Representative H&E staining of livers from sham and ADX mice. **(E)** Representative Oil red O staining of livers from sham and ADX mice.

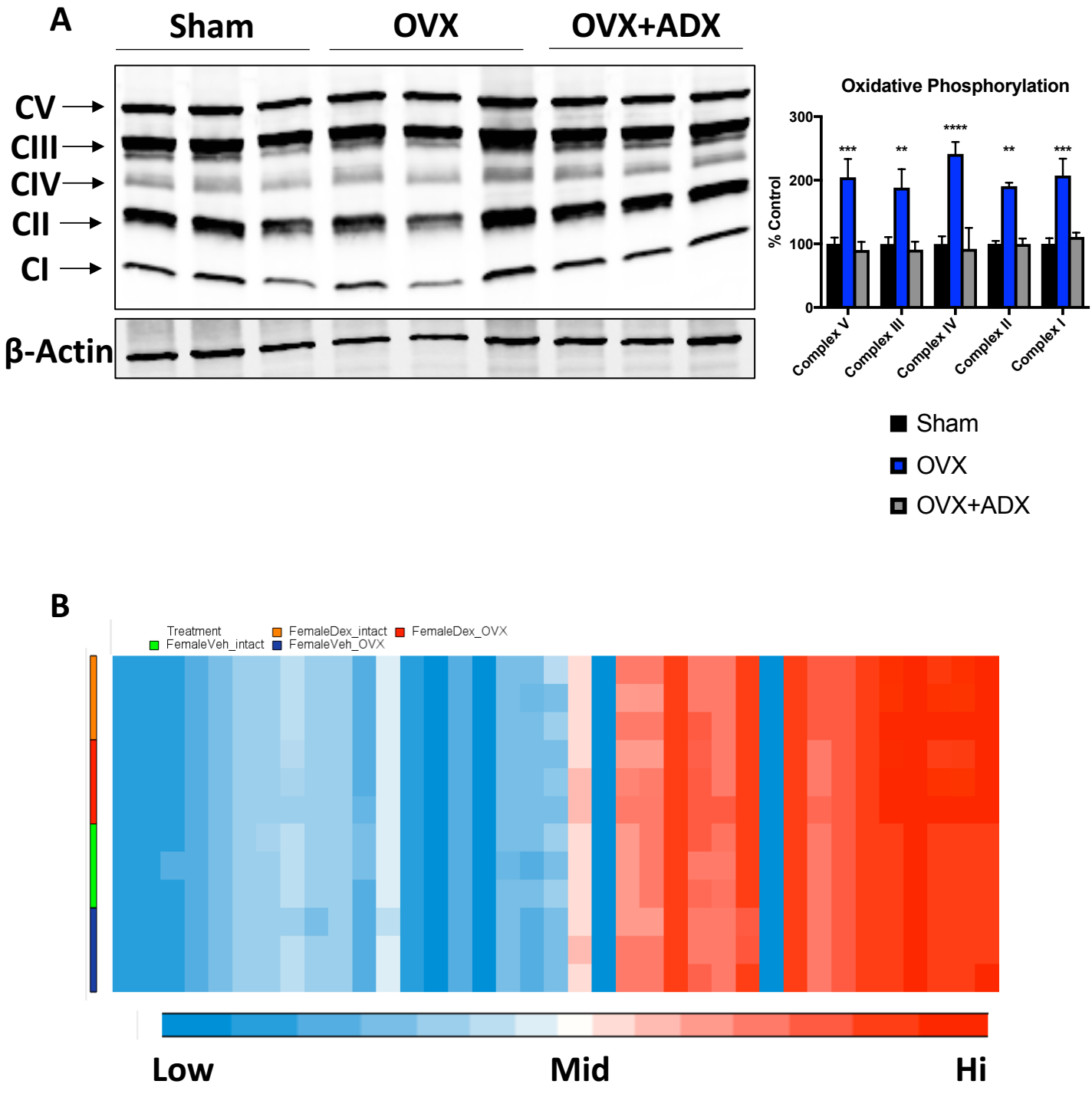

Figure S2: Estrogen deficiency does not promote hepatic steatosis via altered mitochondrial function. Related to Figures 1&2. (A) Oxidative phosphorylation complex components in sham, OVX and OVX+ADX mice with quantification on the right. N=3 mice per group. ** denotes *p<0.01,* *** *p<0.001,* **** *p<0.0001* compared to sham mice. **(B)** Heatmap of RPKM values from RNA-Seq datasets of ChrM RNA counts in normal and hypogonadal female mice treated with dexamethasone.

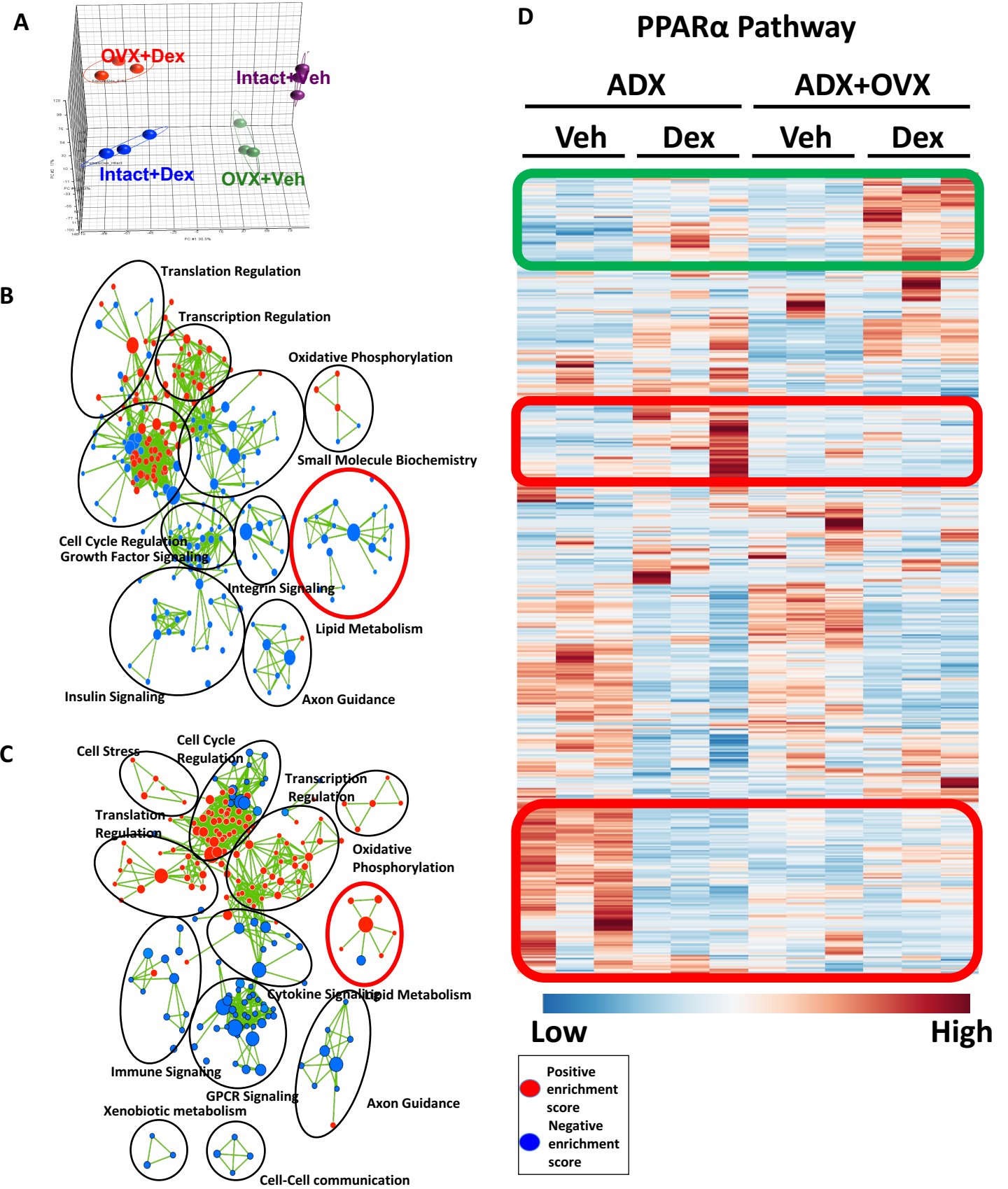

Figure S3: Estrogen deficiency reprograms GR-governed lipid metabolism pathways. Related to Figure 2. **(A)** PCA of RNA-Seq data**. (B)** Gene network analysis of dexamethasone-regulated networks in ovary intact mice. **(C)** Gene network analysis of dexamethasone-regulated networks in ovariectomized mice. Red nodes indicate positive enrichment scores while blue nodes represent negative enrichment scores. **(D)** Heatmap of the PPARα pathway in veh and dexamethasone treated adx and adx+ovx mice.

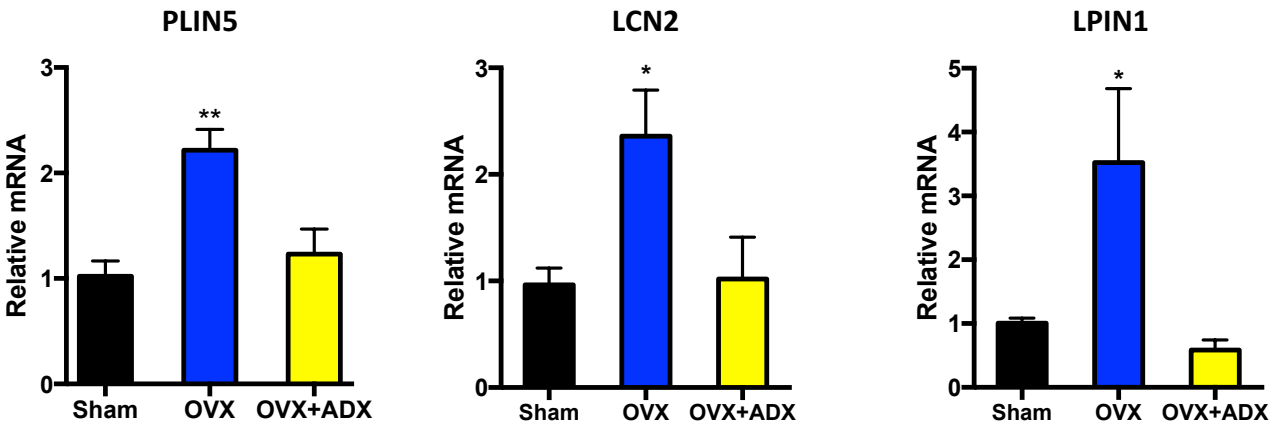

Figure S4: Estrogen deficiency upregulates PLIN5, LCN2 and LPIN1 in a glucocorticoid-dependent manner. Related to Figure 2. PLIN5, LCN2 and LPIN1 are aberrantly expressed in hypogonadal female mice in a GC-dependent manner. PLIN5, LCN2 and LPIN1 mRNA expression in sham, OVX'd and OVX'd+ADX'd mice. N=3-5 per group. Data are expressed as relative mRNA normalized to PPIB mRNA ± SEM. * denotes $p<0.05$, ** $p<0.01$.

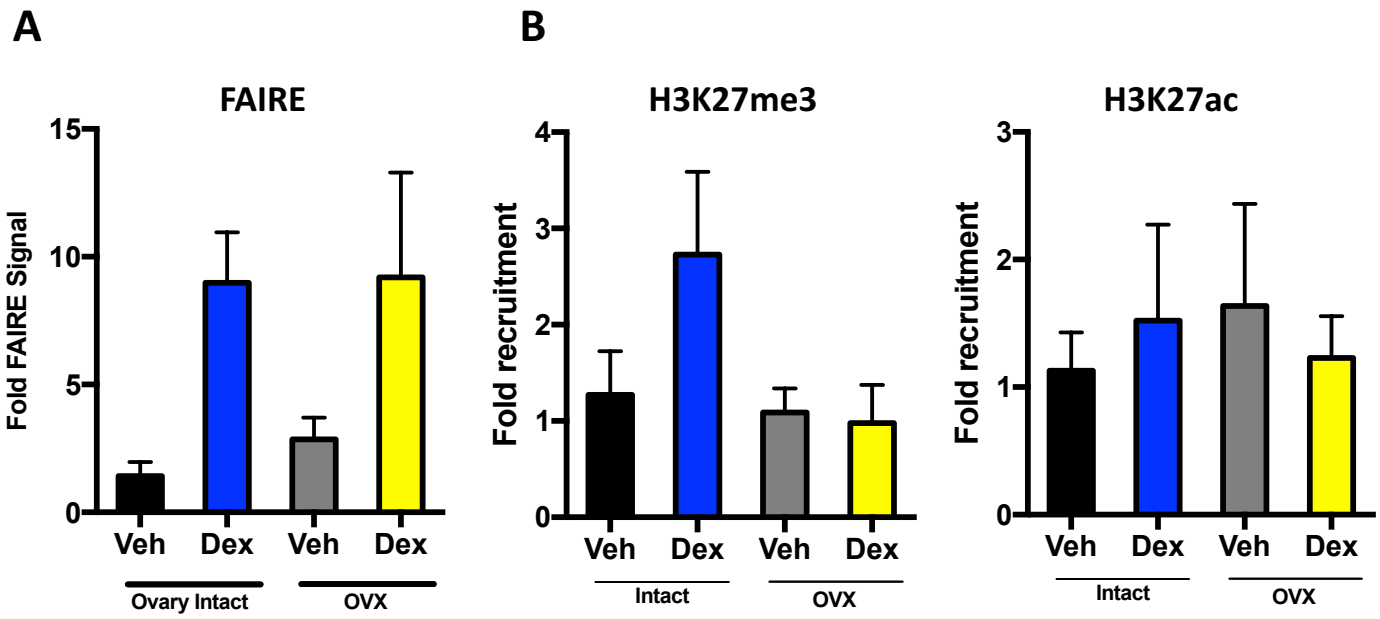

Figure S5: Altered chromatin architecture and epigenetic marks do not underlie OVX-induced glucocorticoid hypersensitivity. Related to Figure 3. (A) Formaldehyde-assisted isolation of regulatory elements assay in vehicle and dexamethasone-treated ovary intact and ovariectomized mice. Data are expressed as fold FAIRE signal of vehicle treated ovary intact mice. N=4 independent experiments. **(B)** H3K27me3 and H3K27ac ChIP in ovary intact and OVX mice treated with vehicle and dexamethasone. Data are expressed as fold recruitment to vehicle. N=4 independent experiments.

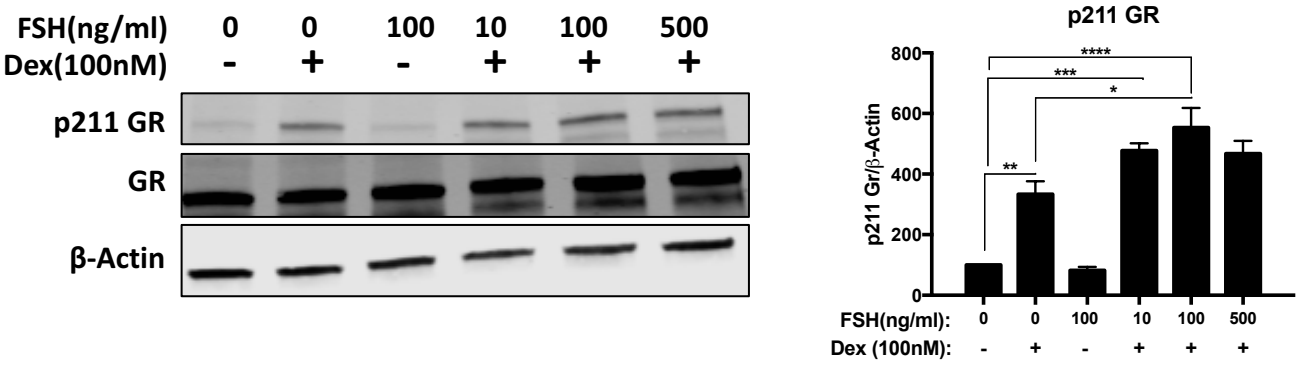

Figure S6: FSH enhances ligand dependent Serine 211 phosphorylation of GR in hepatocytes *in* vitro. Related to Figure 6. In *vitro* regulation of GR phosphorylation by FSH. Western blot detection of p211 GR and total GR in Hepa1-6 murine hepatocytes from vehicle, dexamethasone, and FSH+dexamethasone treated cells. N=3 independent experiments. * denotes $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Gene	*Fold Change ADX*	*Fold Change ADX+OVX*	*# of Putative GREs (Predicted by Jaspar)*
PPARD	**1.28**	**1.56**	**19**
HNF4a	**.986**	**2.48**	**20**
TUT1	**1.44**	**1.58**	**20**
LPIN1	**2.74**	**32.38**	**27**
NR1H3	**1.47**	**1.54**	**18**
PPARGC1a	**1.76**	**2.38**	**14**
ANGPTL4	**1.49**	**3.36**	**27**
AR	**---**	**3.45**	**23**
AGPAT2	**1.53**	**2.14**	**12**

Table S1: Glucocorticoid hypersensitive lipogenic genes in hypogonadal female mice. Related to Figure 2. Hypersensitive glucocorticoid-regulated lipogenic genes are enriched with multiple glucocorticoid response elements within their loci.

Supplemental Experimental Procedures

Materials

Hepa1-6 murine hepatocytes were purchased from ATCC (Manassas, VA) and cultured in high glucose DMEM supplemented with fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA). Twenty-four hours prior to hormone treatment cells were switched to media containing charcoal-dextran stripped fetal bovine serum (Hyclone, Marlsborough, MA).

Quantitative PCR

One hundred nanograms of total RNA was reverse transcribed and amplified using the iScript One-Step RT-PCR kit for probes (Biorad, Hercules, CA). Quantitative real-time PCR (qPCR) was performed with the Biorad CFX96 sequence detection system using predesigned primer/probe sets against ERa, AGPAT6, ELOVL3, CD36 and PPIB from Applied Biosystem (Foster City, CA). Relative fluorescent signal was normalized to PPIB using the ΔCT method.

Chromatin Immunoprecipitation Assay

Approximately 100mg of liver from vehicle and dexamethasone-treated mice was cross- linked and isolated nuclei were subjected to sonication (15 cycles on high, 30 seconds on, 30 seconds off; Diagenode Bioruptor, Denville, NJ). Sonicated DNA was immunoprecipitated with rabbit anti-H3K27ac or anti-H3K27me3 monoclonal antibody (Cell Signaling, Danvers, MA), followed by isolation using the MagnaChIP kit (Millipore, Billerica, MA). Isolated DNA was purified via the QIAquick PCR purification kit (Qiagen, Valencia, CA) and eluted in 50ul of elution buffer. The following primers were designed for GREs within the PLIN5 loci: Forward: CAGCTGCGAGAGGACATT, Reverse: CCCACTGCAAGCTCTGT

Formaldehyde Assisted Isolation of Regulatory Elements Assay

To survey chromatin architecture accessibility FAIRE analysis was performed. Sonicated nuclei from ChIP experiments were subject to phenol: chloroform extraction as previously described (Simon et al., 2013). ChIP primers for PLIN5 GRE described above were used to assess the relative accessibility of that loci via qPCR.

Western blotting and Immunohistochemistry

Protein lysates were prepared from livers of mice by homogenization in SDS-sample buffer (Biorad, Hercules, CA) containing beta-mercaptoethanol (Sigma). Approximately thirty micrograms of total protein was resolved on a 4-20% Tris-glycine gel (Biorad) and transferred onto 0.2uM nitrocellulose membrane (Biorad). Membranes were blocked with Licor blocking buffer (Lincoln, NE) and incubated overnight with anti-estrogen receptor (Abcam) or anti-β-actin (Millipore). ER was used at 1:1000 and β-actin was used at 1:10000. Protein was detected via fluorescent secondary antibody detection (1:10000) (Licor) and imaged on the Licor Odyssey (Licor). Densitometry was performed on the Licor Odyssey software and β-actin was used to normalize loading.