Supplemental Material

Leptin Resistance in the Ovary of Obese Mice is Associated with Profound Changes in the Transcriptome of Cumulus Cells

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Due to the size of the Supplementary Tables, please click on the following links to download and open the respective Excel-file.

Supplementary Table 1. Read Counts & BW.
https://www.cellphysiolbiochem.com/Articles/000228/SM/Supplementary Table 1.xlsx

Supplementary Table 2. BW & gene correlation.
https://www.cellphysiolbiochem.com/Articles/000228/SM/Supplementary Table 2.xlsx

Supplementary Table 3. DESeq values samples.
https://www.cellphysiolbiochem.com/Articles/000228/SM/Supplementary Table 3.xls

Supplementary Table 4. 4wks.
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Supplementary Table 5. 16wks.
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Supplementary Table 6. 4wk_16wk.
https://www.cellphysiolbiochem.com/Articles/000228/SM/Supplementary Table 6.xlsx

Supplementary Table 7. LEPT.
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Supplementary Table 8. LEPT_4wks.
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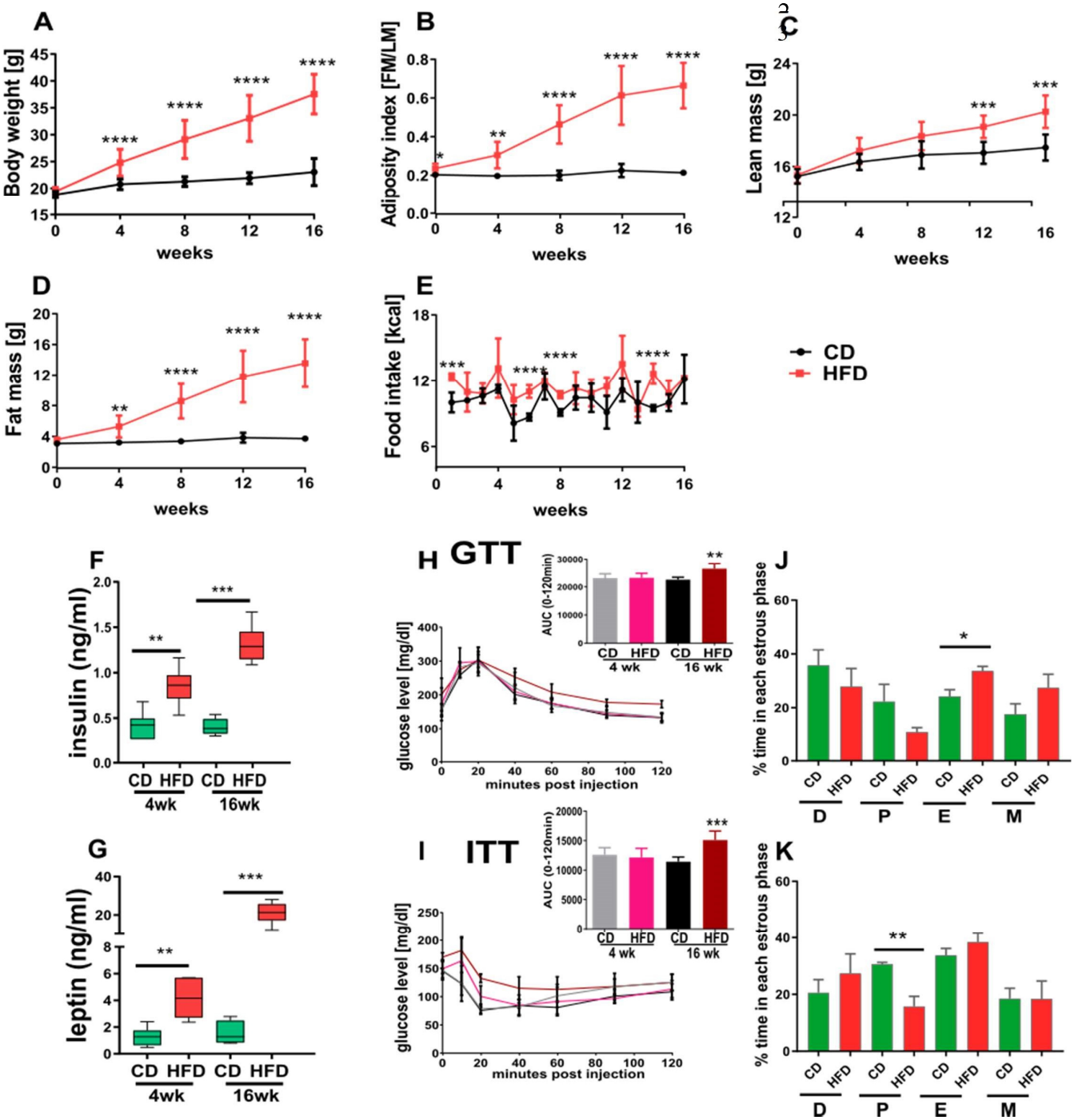
Supplementary Fig. 1. Phenotype characterisation of diet-induced obese mice.

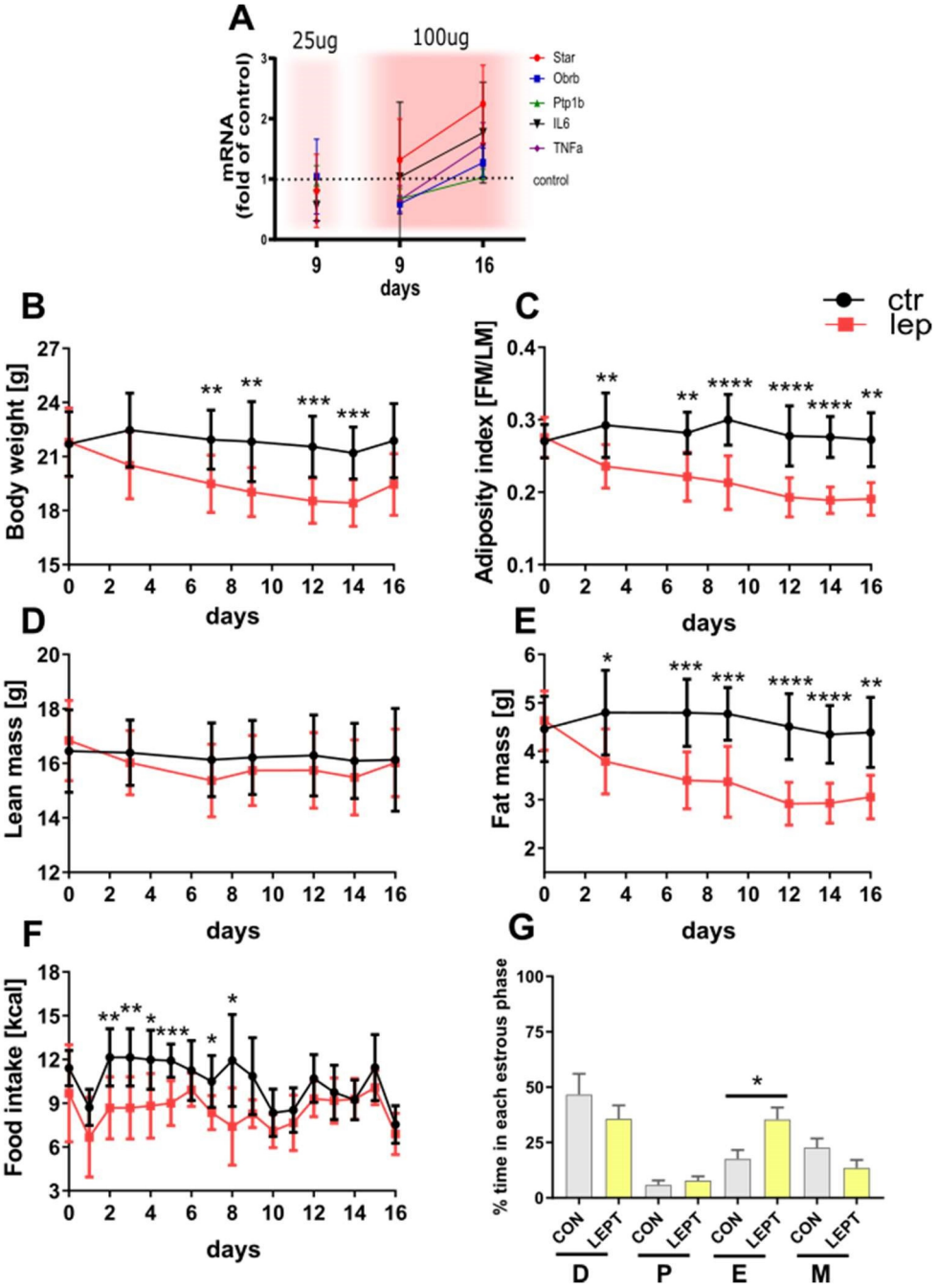
Changes in (A) body weight, (B) adiposity index, (C) lean mass, (D) fat mass, (E) food intake in mice fed chow diet (CD, black line) and high fat diet (HFD, red line) for 4 or 16 weeks (wk). Plasma level of (F) insulin and (G) leptin in mice fed CD or HFD for 4 and 16 wk. Glucose tolerance test (GTT, H) and insulin tolerance test (ITT, I) present glucose levels at 0-120 min after glucose and insulin injection, respectively. Bar graphs in the upper right panel present area under the curve for each group. Grey, pink, black and brown line represent group maintained for 4 wk on CD, 4 wk on HFD, 16 wk on CD and 16 wk on HFD, respectively. Plasma collected from animals in oestrus phase. Proportion of time spent in each oestrous phase of mice subjected to CD or HFD for 4 wk (J) and 16 wk (K) monitored for 12 days. D, dioestrus; E, oestrus; M, metoestrus; P, pro-oestrus. Each bar represents the mean ± SD for n=12. Differences in phenotype characteristics and plasma hormone level between groups were analysed by Mann-Whitney test, oestrous cycle distribution analysed by unpaired t-test. * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Supplementary Fig. 1.



Supplementary Fig. 2. Pharmacologically hyperleptinemic mouse model validation.
In order to validate the length of the treatment and the dose of leptin, we analysed whole ovary mRNA from animals treated with 25 or 100 μg of leptin for 9 or 16 d. Injection of 100 μg for 16 days caused changes in the abundance of leptin-responsive transcripts [69–72] in ovarian extracts collected from animals in oestrous stage. Animals were injected with saline (C) or different doses of leptin (L) for 9 or 16 days (d). (A) mRNA level of *steroidogenic acute regulatory protein (Star), long isoform of leptin receptor Obrb, protein tyrosine phosphatase non-receptor type 1 (Ptp1b), interleukin 6 (Il6), tumor necrosis factor a (Tnfa)* expressed as fold of control after injecting animals for 9 or 16 days with 25μg or 100 μg of leptin. Changes in (B) body weight, (C) adiposity index, (D) lean mass, (E) fat mass, (F) food intake in mice intraperitoneally injected with saline (ctr, black line) or leptin (lep, red line) for 16 days. (G) Proportion of time spend in each oestrous phase of hyperleptinemic mice. D, dioestrus; E, oestrus; M, metoestrus; P, pro-oestrus. Each bar represents the mean ± SD. Differences in phenotype characteristics between groups were analysed by Mann- Whitney test, oestrous cycle distribution analysed by unpaired t-test. Data show mean values for n=10. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

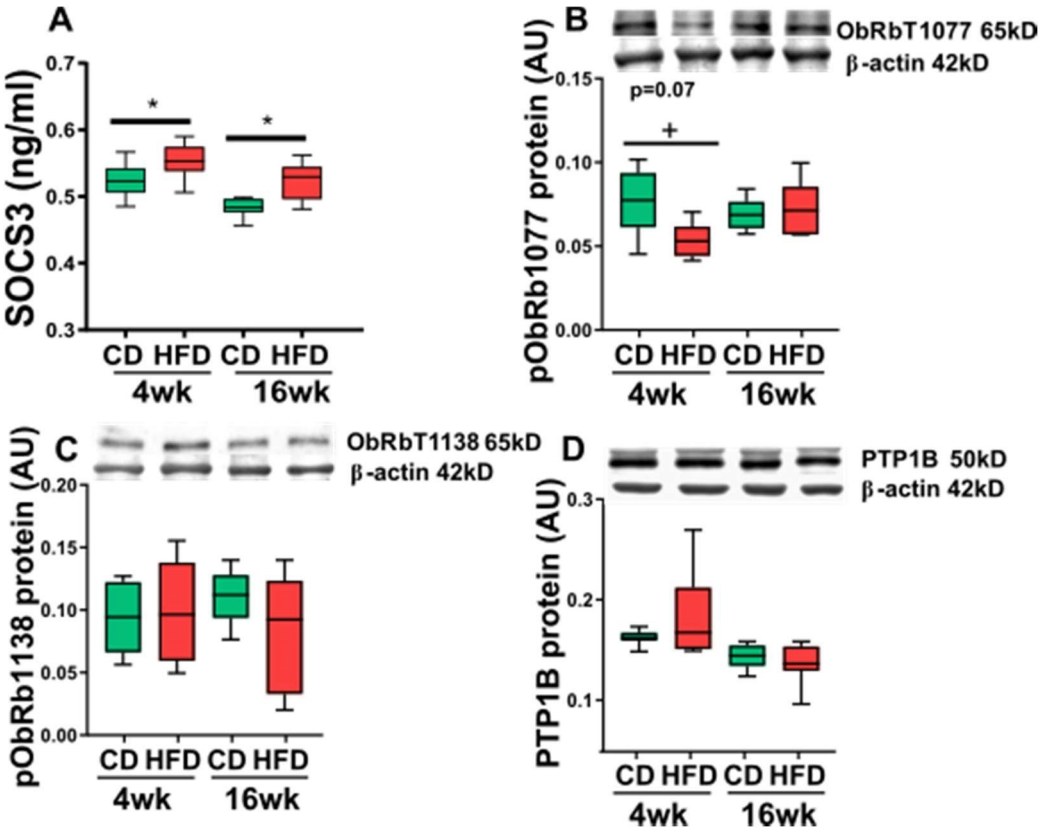
Supplementary Fig. 2.



Supplementary Fig. 3. Expression of leptin signalling components in the ovary of diet induced obese mice.

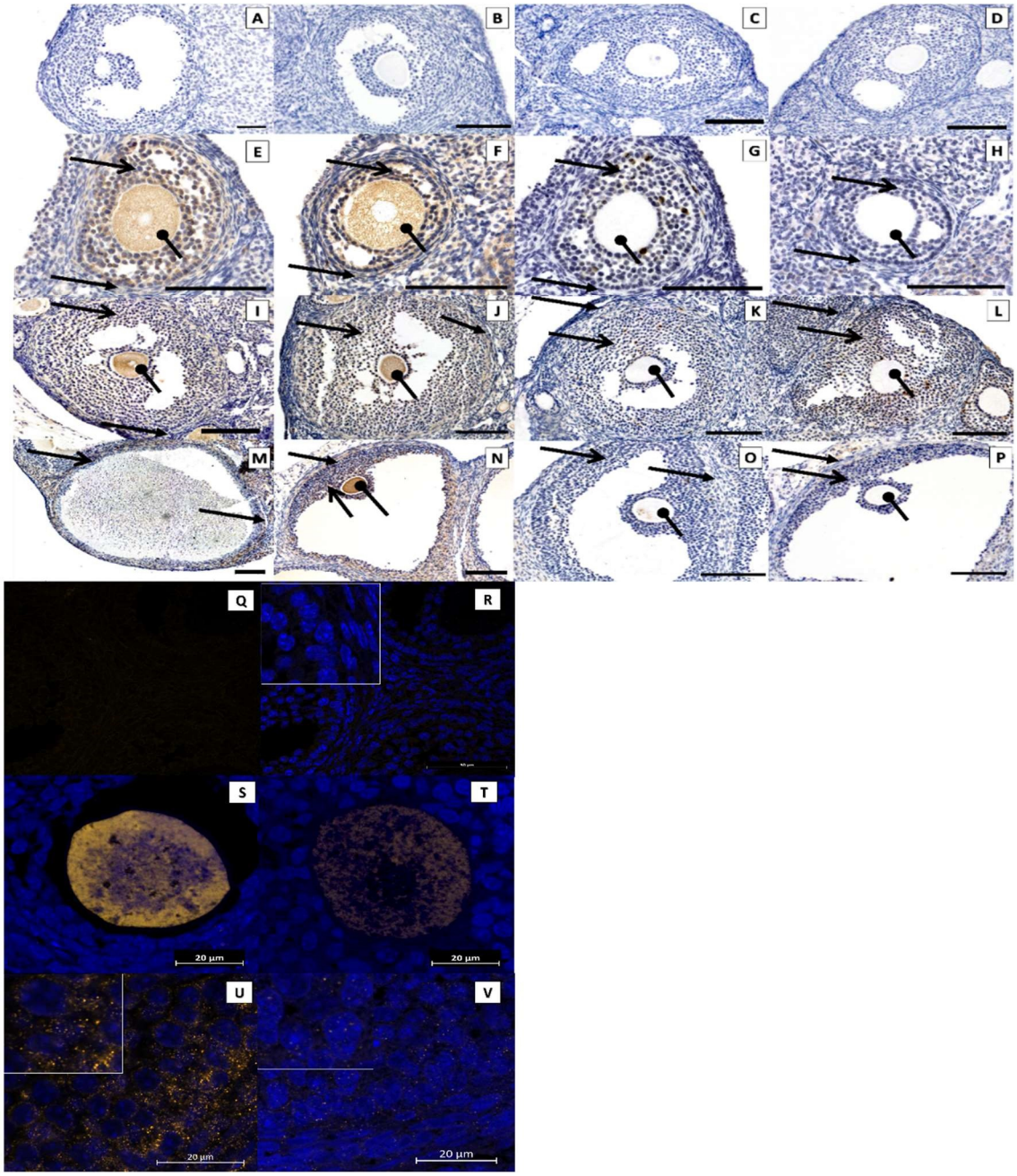
Protein abundance of components of the leptin signalling pathway in ovarian extracts analysed by Western blot or ELISA. Animals were maintained on chow diet (CD) or high fat diet (HFD) for 4 or 16 weeks (wk). (A) SOCS3 ovarian quantification in ELISA test. Phosphorylation of (B) tyrosine 1077 of leptin receptor, (C) tyrosine 1138 of leptin receptor, abundance of (D) PTP1B. Protein expression of β-actin were used to normalize the expression data. Each bar represents the mean ± SD. Differences between groups were analysed by Mann-Whitney test. N=4-8 for immunoblots and N=8 for ELISA. * $p<0.05$; ** $p<0.01$; ***$p<0.001$; + $p=0.07$.

Supplementary Fig. 3.



Supplementary Fig. 4. Immunolocalisation of SOCS3 and PTP1B protein in the ovary. Immunohistochemical localisation of SOCS3 and PTP1B protein during follicle development in mice fed chow diet (CD) or high fat diet (HFD) for 4 and 16 weeks (wk) and intraperitoneally injected with saline (C) or leptin (L) for 16 days (d). Positive staining in brown, counterstaining with heamatoxylin. Negative control stained with polyclonal rabbit IgG (A, B) 4 wk CD, (C, D) 4 wk HFD, localisation in secondary follicle SOCS3 (E) 4 wk CD and (F) 4 wk HFD, PTP1B (G) 4 wks CD, (H) 4 wks HFD, antral follicles SOCS3 (I) 16 wk CD, (J) 16 wk HFD, PTP1B (K) 16 wk CD, (L) 16 wk HFD, preovulatory follicle SOCS3 (M) 16 C, (N) 16 L, PTP1B (O) 16 C, (P) 16 L. The scale bar represents 100μm. The specificity of SOCS3 staining was confirmed by immunofluorescent localisation in *ob/ob* mice with genetic deficiency of leptin. Positive staining in orange, nuclear counterstaining with DAPI in blue. (Q-R) negative control 16 wk CD performed with polyclonal rabbit IgG, SOCS3 localised in (S, T) secondary follicle and (U,V) antral follicle from controls (*ob/ob.* +/+; S,U) and leptin deficient ovaries (*ob/ob* -/-; T,V). Images are representatives of 3 biological replicates. Inserts in left top corners are the amplifications of granulosa cells. Pictures are representatives of 3 biological replicates. The scale bar represents 20μm.

Supplementary Fig. 4.

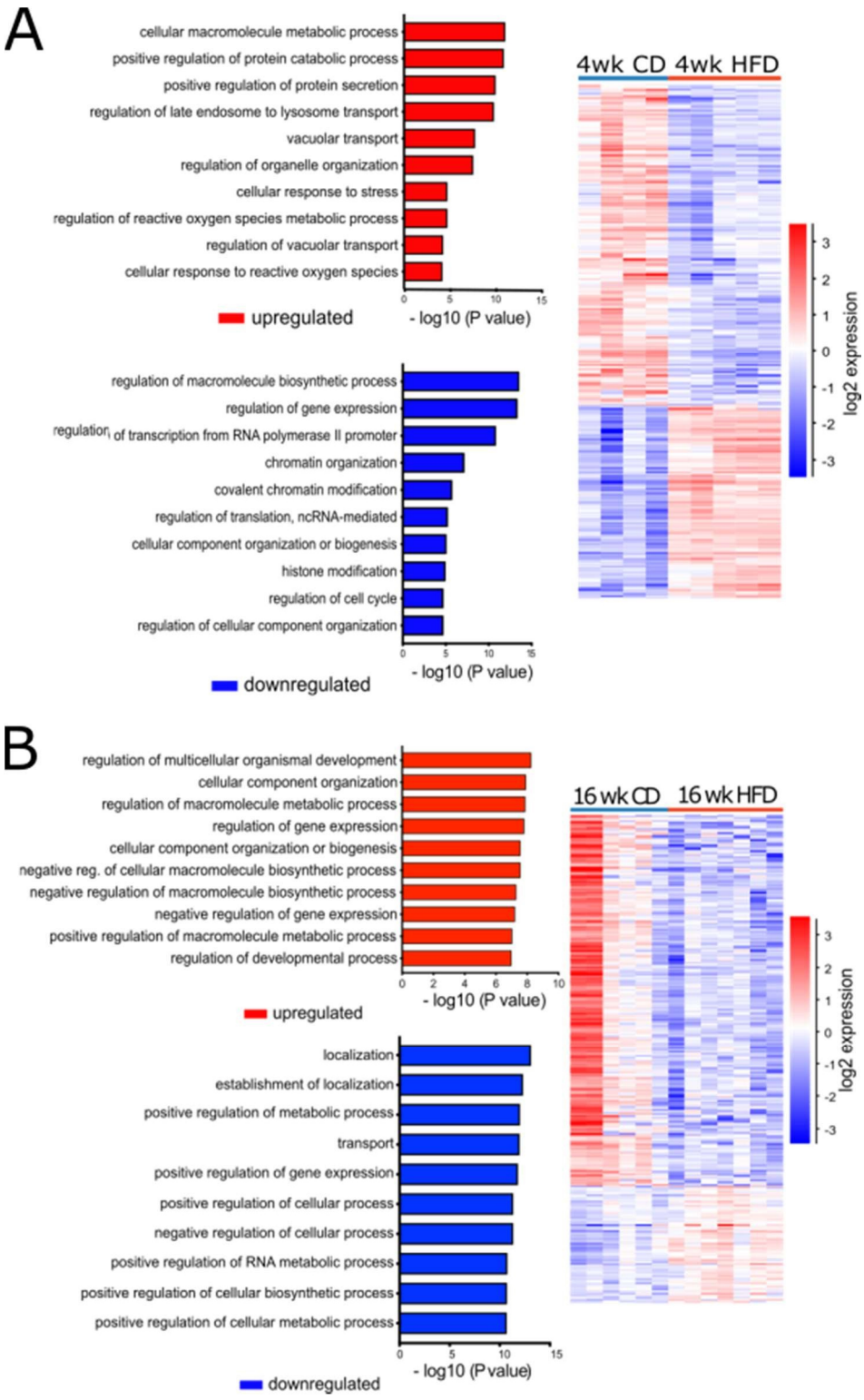


Supplementary Fig. 5. Differentially expressed genes and associated pathways in cumulus cells from diet- induced obesity protocol.

DESeq2 analysis of transcriptome data in cumulus cells obtained from mice after 4 or 16 weeks (wk) of chow diet (CD) or high fat diet (HFD). N= 3-7 mice per group.

On the right heatmap showing hierarchical clustering of (A) 997 differentially expressed genes after submitting mice to 4 weeks of CD and HFD, (B) 846 differentially expressed genes after submitting mice to 16 weeks of CD and HFD. On the left presentation of pathways of genes with the most significant enrichment after gene ontology analysis. Gene ontology analysis performed with Gene Ontology Enrichment Analysis and Visualisation Tool.

Supplementary Fig. 5.

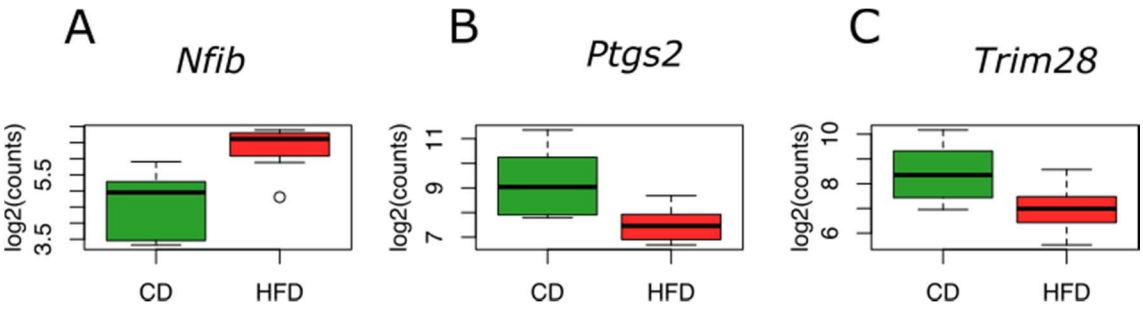


Supplementary Fig. 6. Oocyte competence and embryo quality markers differentially expressed in cumulus cells from mice with late obesity.

DESeq2 analysis of transcriptome data in cumulus cells obtained from mice after 16 weeks of chow diet (CD) or high fat diet (HFD). N= 3-7 mice per group.

Expression of embryo quality markers (A) *nuclear factor I B (Nfib)*, (B) *cyclooxygenase 2 (Ptgs2)* and oocyte competence marker (C) *tripartite motif containing 28 (Trim28).* Log2 of counts.

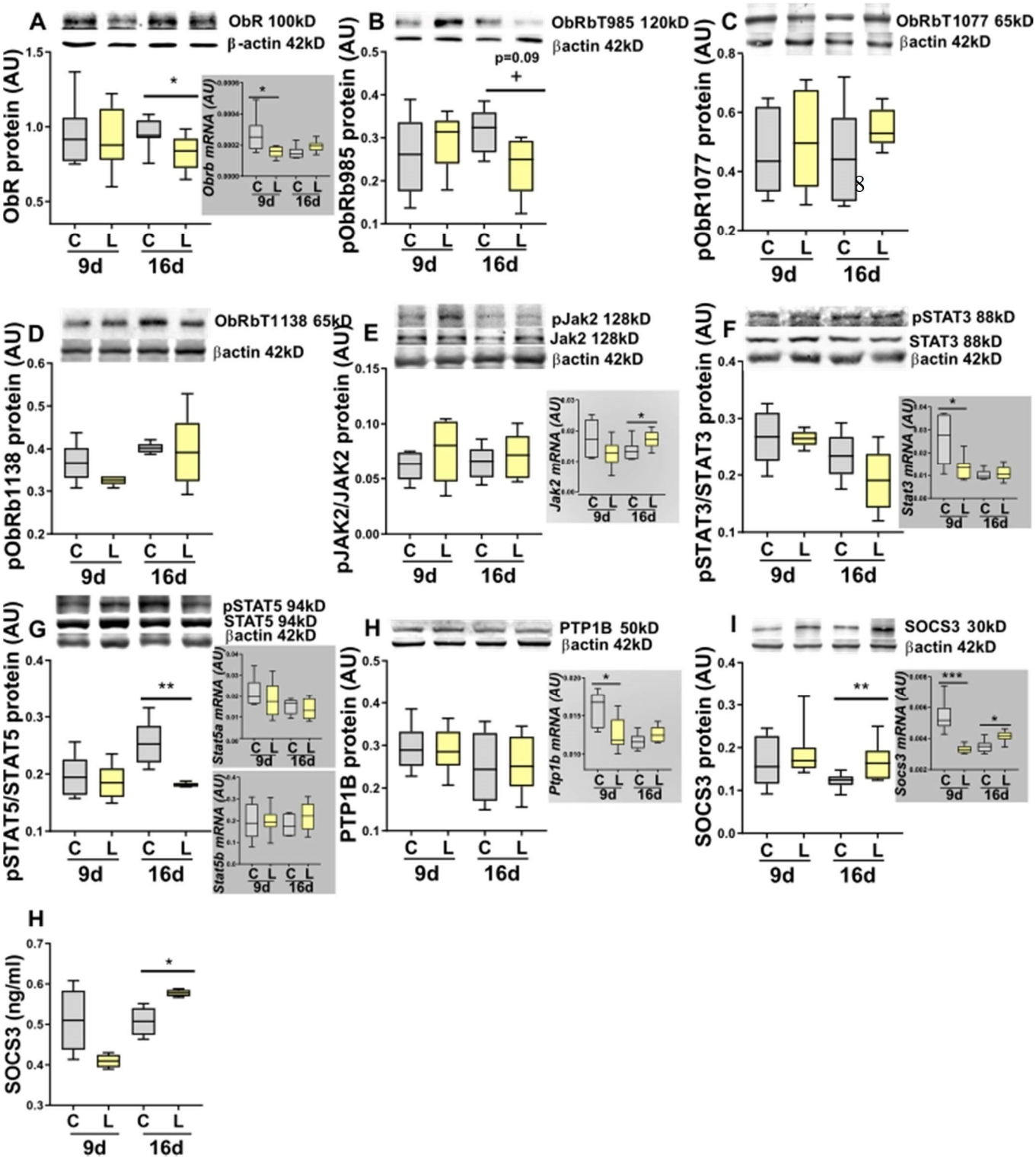
Supplementary Fig. 6.



Supplementary Fig. 7. Expression of leptin signalling components in the ovary of pharmacologically hyperleptinemic mice.

Abundance of mRNA (grey box) and protein of leptin signalling pathway components in ovarian extracts collected from animals injected with saline (C) or 100 μg of leptin (L) for 9 or 16 days (d) and sacrificed in oestrus stage. Expression of (A) leptin receptor (ObR), phosphorylation of (B) tyrosine 985 of leptin receptor, (C) tyrosine 1077 of leptin receptor, (D) tyrosine 1138 of leptin receptor, (E) Janus kinase 2 (JAK2), (F) signal transducer and activator of transcription 3 (STAT3), (G) STAT5, expression of (H) protein tyrosine phosphatase 1B (PTP1B) and (I) suppressor of cytokine signalling 3 (SOCS3) determined by real-time PCR and Western blot. (J) SOCS3 ovarian quantification in animals in oestrus stage determined by ELISA. mRNA expression of *Rpl37* and protein expression of β-actin was used to normalize the expression data. Each bar represents the mean ± SD. Differences between groups were analysed by Mann- Whitney test. N=4-8 for immunoblots and N=8 for RT PCR analysis and ELISA. * $p<0.05$; ** $p<0.01$; ***$p<0.001$; + $p=0.09$.

Supplementary Fig. 7.

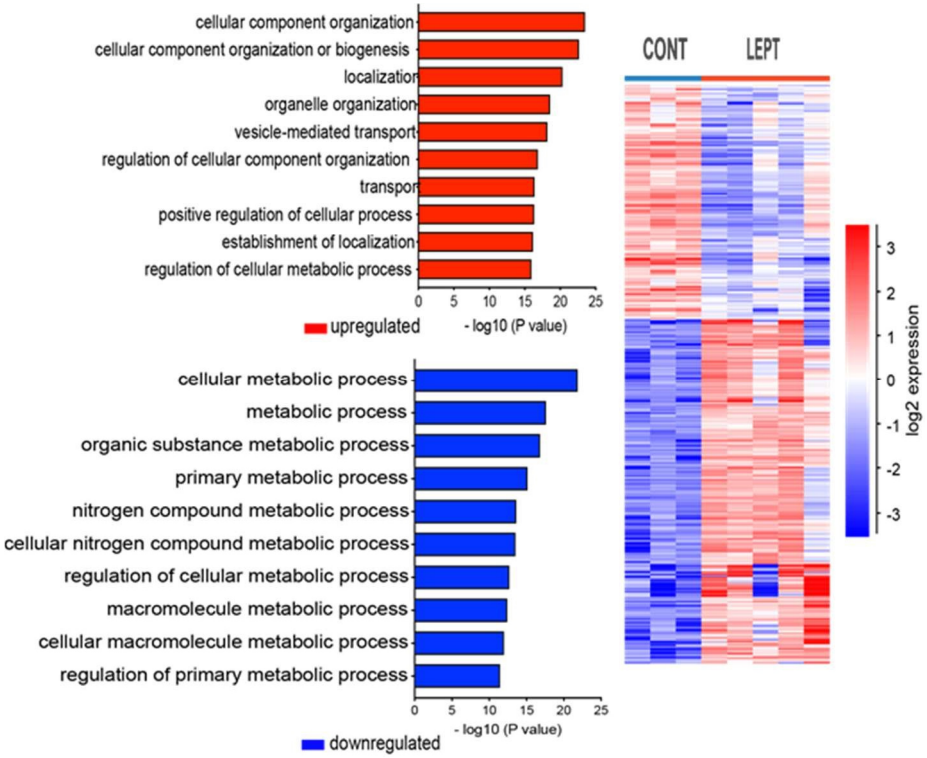


Supplementary Fig. 8. Differentially expressed genes and associated pathways in cumulus cells from hyperleptinemic mice.

DESeq2 analysis of transcriptome data in cumulus cells obtained from mice treated with saline (CONT) and leptin (LEPT). N= 3-7 mice per group.

On the right heatmap showing hierarchical clustering of 2026 differentially expressed genes after treating mice with leptin for 16 days. On the left presentation of pathways of genes with the most significant relevance after gene ontology analysis. Gene ontology analysis performed with Gene Ontology Enrichment Analysis and Visualisation Tool.

Supplementary Fig. 8.



Supplementary Fig. 9. Similarities between profiles of genes differentially expressed in cumulus cells in diet induced- obese mice and leptin treated mice.

DESeq2 analysis of transcriptome data in cumulus cells (CC) obtained from mice treated with saline (CONT) and leptin (LEPT) or after 4 or 16 weeks (wk) of chow diet (CD) or high fat diet (HFD). N= 3-7 mice per group. Heatmaps representing fold change in expression of genes associated with the following pathways or processes: (A) leptin signalling, (B) tricarboxylic acid (TCA) cycle, (C) oocyte competence, (D) genes regulated by oocyte-derived growth differentiation factor (GDF) 9, (E) inflammation, oxidative stress and endoplasmic reticulum stress, (F) DNA damage and apoptosis in CC. log2_FC of reads per million (RPM).

Supplementary Fig. 9.