

**Title: Genistein and Sex Hormone Supplementation Modulated Hepatic PPAR $\alpha$ ,  $\delta$  and  $\gamma$  Subtypes and STAT1 Expressions in a NASH Rat Model with Bilateral Orchidectomy**

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**Type of manuscript: Original Research Article**

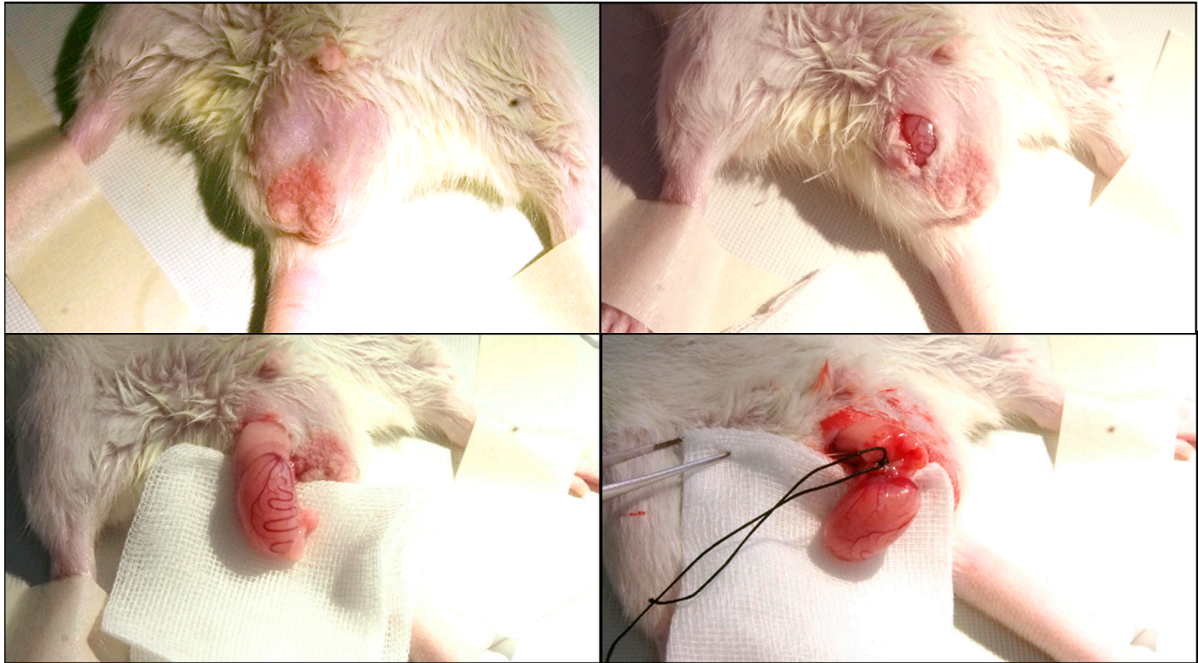
**Supplementary Table S1.** Specific primers sequence for gene expression analysis.

Gene	Function	Nucleotide sequence
FAS	Forward reverse	5'-ACC-TCA-TCA-CTA-GAA-GCC-ACC-AG-3' 5'-GTG-GTA-CTT-GGC-CTT-GGG-TTT-A-3'
SREBP1c	Forward reverse	5'-CTG-TCG-TCT-ACC-ATA-AGC-TGC-AC-3' 5'-ATA-GCA-TCT-CCT-GCA-CAC-TCA-GC-3'
$\beta$ -actin	Forward reverse	5'-ACG-GTC-AGG-TCA-TCA-CTA-TCG-3' 5'-GGC-ATA-GAG-GTC-TTT-ACG-GAT-G-3'

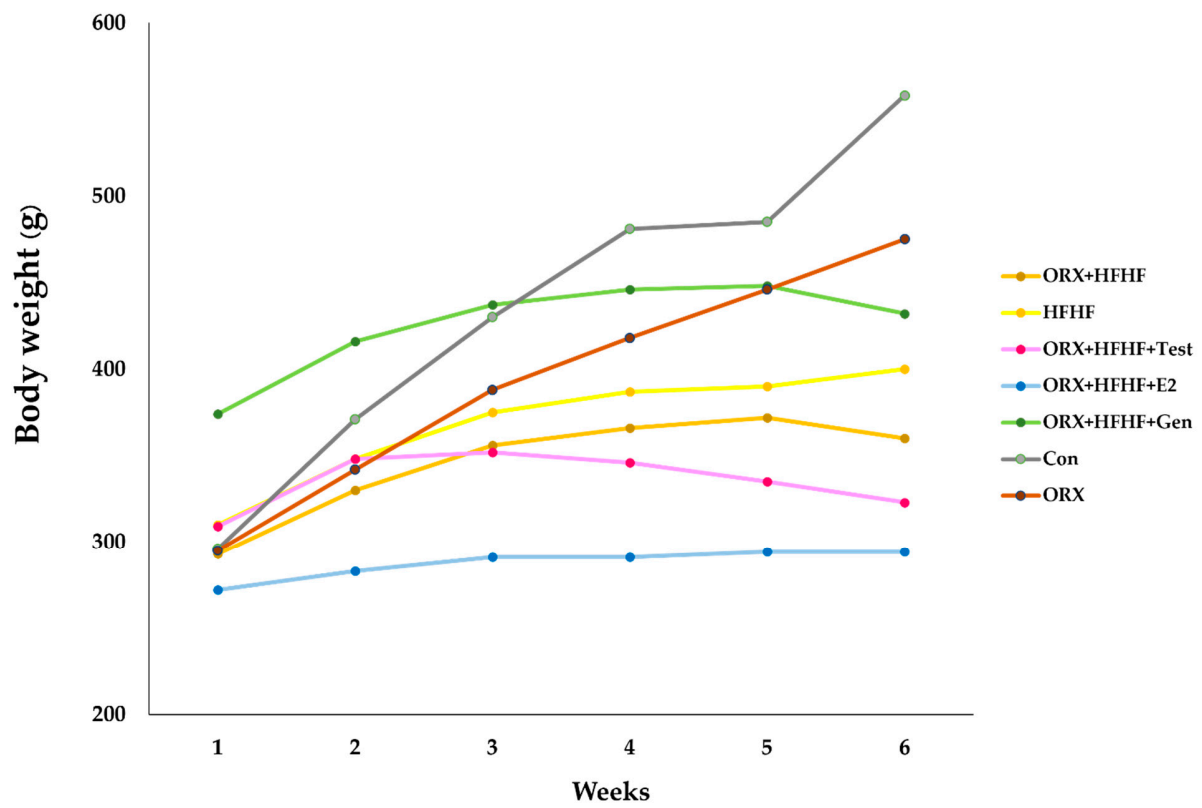
**Supplementary Table S2.** The quantification data of Oil Red O staining area presented as a percentage in all experimental groups.

Groups Lipid deposition	Control	ORX	ORX+HFHF	HFHF	ORX+HFHF+ Test	ORX+HFHF+ E2	ORX+HFHF+ Gen
Percentage of positive Oil Red O staining area	2.80±0.98	6.68±1.30 <sup>a</sup>	54.19±1.88 <sup>a,b</sup>	26.9±4.20 <sup>a,b,c</sup>	2.86±0.36 <sup>b,c,d</sup>	2.03±0.70 <sup>b,c,d</sup>	10.77±2.99 <sup>a,b,c,d</sup>

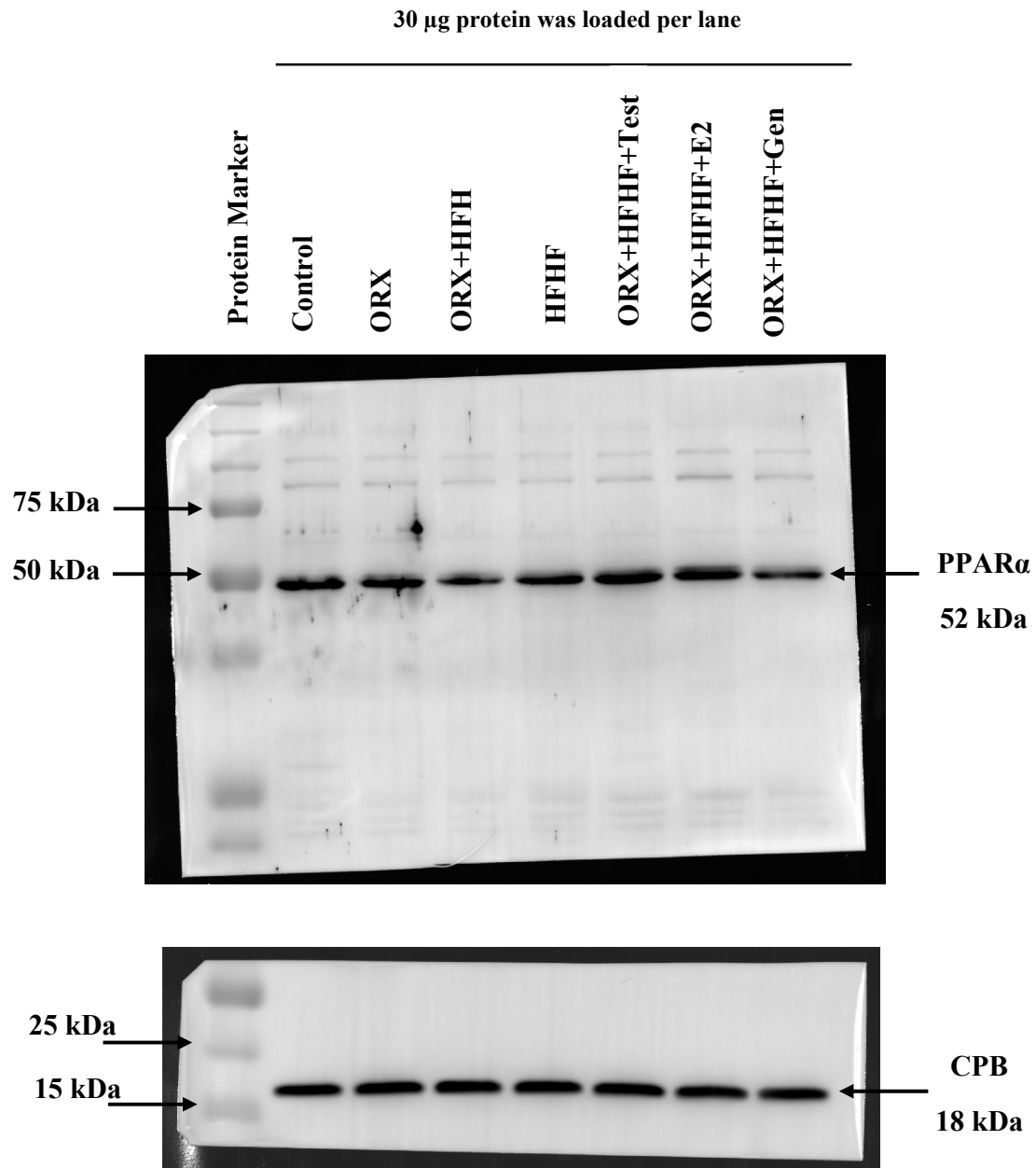
Data is expressed as mean ± SD. a: p<0.05 when compared with the control group, b: p<0.05 when compared with the ORX group, c: p<0.05 when compared with the ORX+HFHF group, d: p<0.05 when compared with the HFHF group.



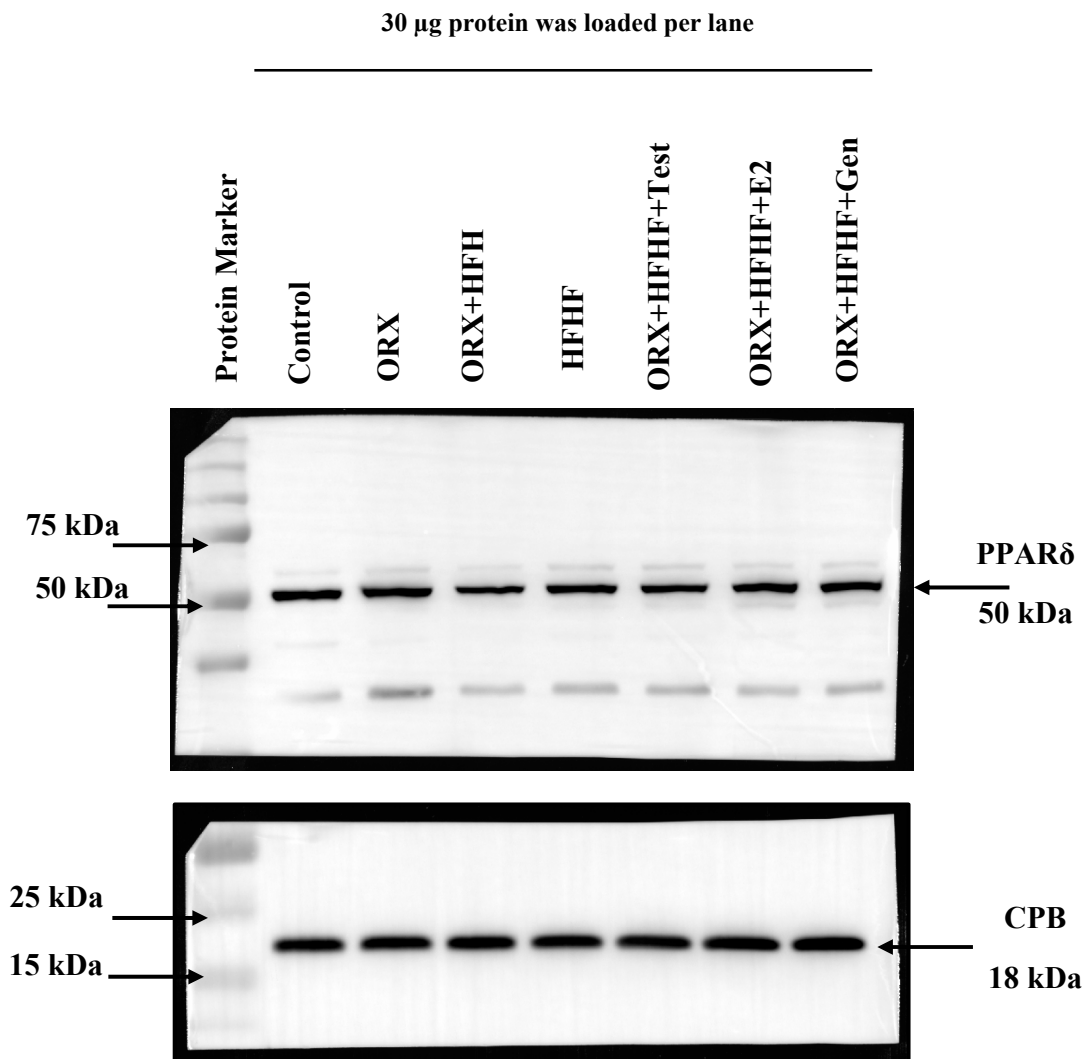
**Supplementary Fig S1.** The bilateral orchidectomy procedure was performed to induce the state of testosterone deficiency in orchidectomized groups.



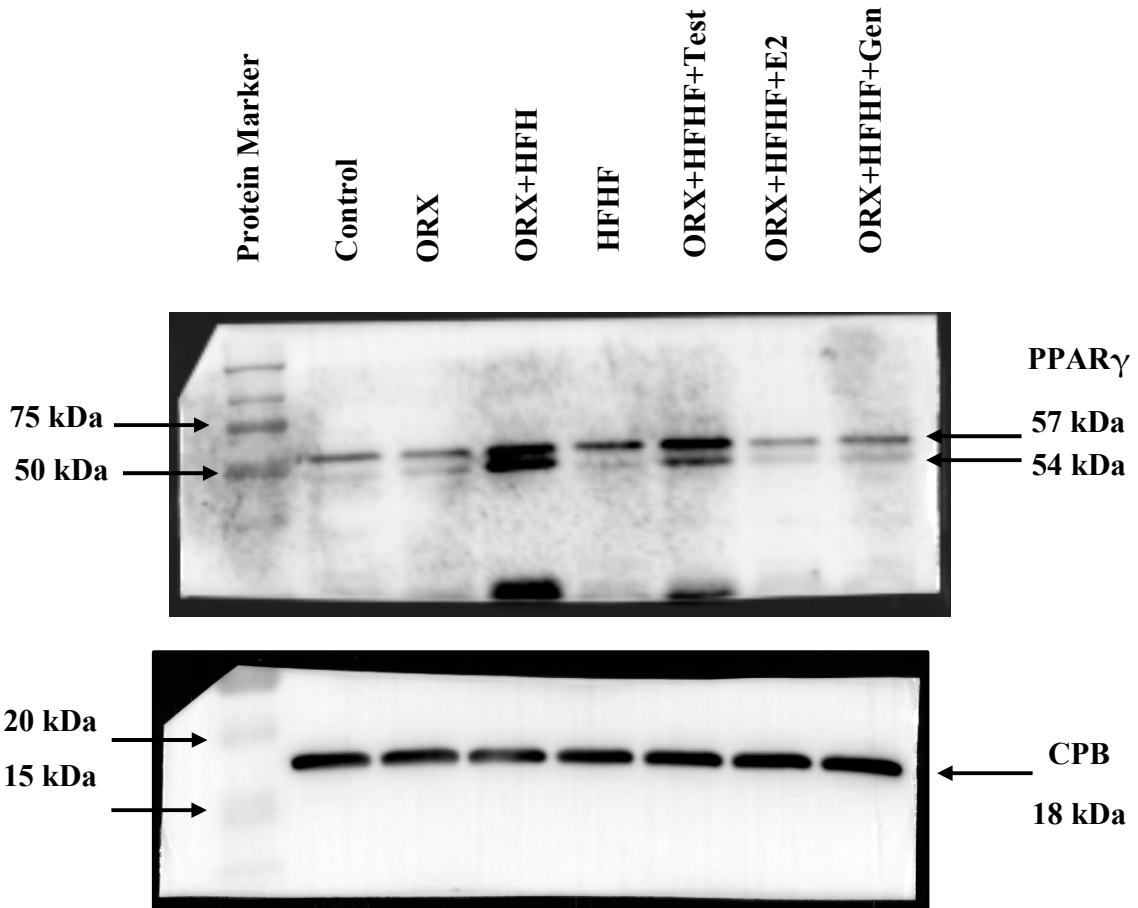
**Supplementary Fig S2.** Weekly mean body weight growth curves of the rats in all experimental groups (n=6 per group, except HFHF group, n=5).



**Supplementary Fig S3.** Immunoblotting images for PPAR $\alpha$  and cyclophilin B (CPB) band densities. Seven panels (Left to right side) indicate each experimental group. The first four panels represent untreated groups; control, ORX, ORX+HFHF and HFHF groups, respectively and the last three panels represent all treated groups; ORX+HFHF+Test, ORX+HFHF+E2 and ORX+HFHF+Gen groups, respectively). Abbreviations: Test, Testosterone; E2, estradiol; Gen, Genistein.

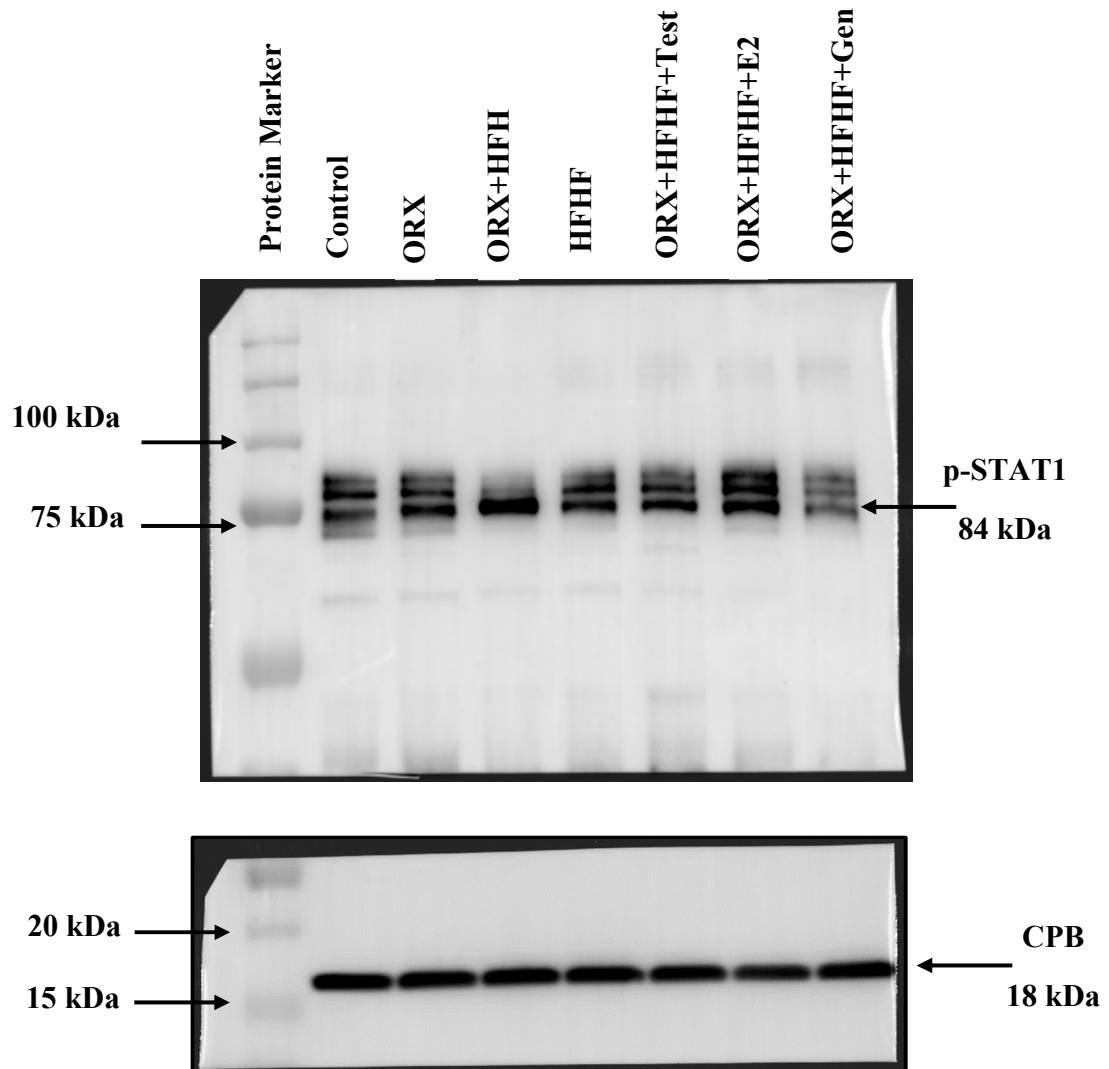


**Supplementary Fig S4.** Immunoblotting images for PPAR $\delta$  and cyclophilin B (CPB) band densities. Seven panels (Left to right side) indicate each experimental group. The first four panels represent untreated groups; control, ORX, ORX+HFHF and HFHF groups, respectively and the last three panels represent all treated groups; ORX+HFHF+Test, ORX+HFHF+E2 and ORX+HFHF+Gen groups, respectively). Abbreviations: Test, Testosterone; E2, estradiol; Gen, Genistein.

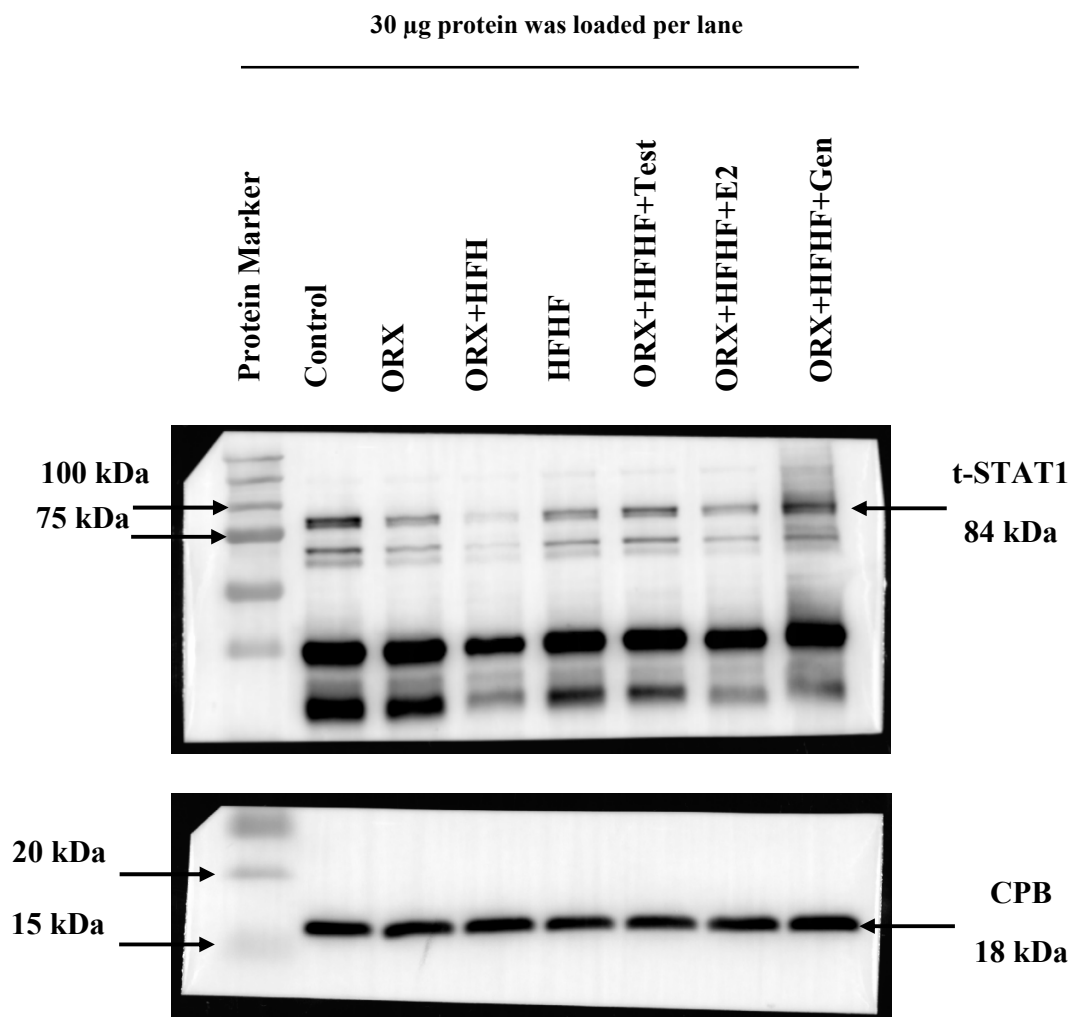


**Supplementary Fig S5.** Immunoblotting images for PPAR $\gamma$  and cyclophilin B (CPB) band densities. Seven panels (Left to right side) indicate each experimental group. The first four panels represent untreated groups; control, ORX, ORX+HFHF and HFHF groups, respectively and the last three panels represent all treated groups; ORX+HFHF+Test, ORX+HFHF+E2 and ORX+HFHF+Gen groups, respectively). Abbreviations: Test, Testosterone; E2, estradiol; Gen, Genistein.

30  $\mu$ g protein was loaded per lane







**Supplementary Fig S6.** Immunoblotting images for p-STAT1 and t-STAT1, and cyclophilin B (CPB) band densities. Seven panels (Left to right side) indicate each experimental group. The first four panels represent untreated groups; control, ORX, ORX+HFHF and HFHF groups, respectively and the last three panels represent all treated groups; ORX+HFHF+Test, ORX+HFHF+E2 and ORX+HFHF+Gen groups, respectively). Abbreviations: Test, Testosterone; E2, estradiol; Gen, Genistein.