Supplementary materials

Text S1 Behavioral indexes of copulatory test

A series of behavioral indexes recorded in the present study were listed as follow[1]: Capture times (CT) are the number of the male mouse captures the female mouse; Mounting latency (ML) means the time taken for the first mount following the introduction of female; Mounting frequency (MF) is the total number of mounting; Intromission latency (IL) is the time taken for first intromission following introduction of the female; Intromission frequency (IF) is the total number of intromission; Ejaculation latency (EL) is the time interval between first mount with pelvic thrusting and vaginal penetration to ejaculation; Ejaculation frequency (EF) means the total number of ejaculation; Post-ejaculatory intervals (PEI) are the interval between first ejaculation and the following intromission. After sexual behavior test, the serum of male mice was obtained to measure the related biochemical parameters. Testis, seminal vesicle, epididymis, and penis tissue etc were collected and weighted, and one side of testis was stored at 10% formalin and the another part were collected for histological analysis and biochemical, respectively. All female mice were euthanized after experiment.

Text S2 Hematoxyli-eosin (HE) staining, immunohistochemistry (IHC) and immunoflourescence (IF)

The harvested testis, epididymis, and liver tissues were fixed in 4% paraformaldehyde solution for 48 h and embedded in paraffin. Then the tissues were sliced into 5 μm sections, and examined by hematoxylin-eosin (HE) staining. The stained slides were observed using a microscope (Leica, Germany).

For IHC assay, testis tissues were fixed and embedded in paraffin. In brief, paraffin sections were incubated with primary antibodies against StAR (1:100 dulution, Proteintech Group, USA) and HSD17B3 (1:100 dulution, Proteintech Group, USA) for overnight at 4 ℃. Subsequently, the tissue sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:4000). Then the sections were washed with phosphate buffered saline (PBS) and added with 3,3-diaminobenzidine tetrahydrochloride Dihydrate (DAB) to react for 10 min at room temperature. All sections were counterstained with hematoxylin. Immunostaining was observed using a microscope (Leica, Germany) and captured by a slice scanner (PANNORAMIC, 3DHISTECH).

For the IF assay, testis tissue sections were blocked with 1% bovine serum albumin and then incubated with primary antibodies CYP17A1 (1:200, Proteintech Group, USA) for overnight at 4 ℃. The sections were then incubated with fluorescence-conjugated secondary antibodies at room temperature for 2 h. Finally, the nuclei of cells were stained by 4',6-diamidino-2-phenylindole (DAPI). Images were taken under a fluorescence microscope (Nikon, Japan). The final score was calculated by multiplying the intensity scores with staining range using ImagePro Plus 6 software.

Text S3 Untargeted Metabolomics Methods

(1) Sample Collection and Preparation

Each aliquot (150 μL) of the serum sample was stored at –80 °C until UPLC-Q-TOF/MS analysis. The serum samples were thawed at 4°C and 100 μL aliquots were mixed with 400 μL of cold methanol/acetonitrile (1:1, v/v) to remove the protein. The mixture was centrifuged for 15 min (14000g, 4°C). The supernatant was dried in a vacuum centrifuge. For LC-MS analysis, the samples were re-dissolved in 100 μL acetonitrile/water (1:1, v/v) solvent. To monitor the stability and repeatability of instrument analysis, quality control (QC) samples were prepared by pooling 10 μL of each sample and analyzed together with the other samples. The QC samples were inserted regularly and analyzed in every 5 samples.

(2) LC-MS/MS Analysis

Untargeted metabolomics samples were performed using an UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex TripleTOF 6600).

For HILIC separation, samples were analyzed using a 2.1 mm × 100 mm ACQUIY UPLC BEH 1.7 μm column (waters, Ireland). In both ESI positive and negative modes, the mobile phase contained A = 25 mM ammonium acetate and 25 mM ammonium hydroxide in water and B= acetonitrile. The gradient was 85% B for 1 min and was linearly reduced to 65% in 11 min, and then was reduced to 40% in 0.1 min and kept for 4 min, and then increased to 85% in 0.1 min, with a 5 min re-equilibration period employed.

The ESI source conditions were set as follows: Ion Source Gas1 (Gas1) as 60, Ion Source Gas2 (Gas2) as 60, curtain gas (CUR) as 30, source temperature: 600℃, IonSpray Voltage Floating (ISVF) ± 5500 V. In MS only acquisition, the instrument was set to acquire over the m/z range 60-1000 Da, and the accumulation time for TOF MS scan was set at 0.20 s/spectra. In auto MS/MS acquisition, the instrument was set to acquire over the m/z range 25-1000 Da, and

the accumulation time for product ion scan was set at 0.05 s/spectra. The product ion scan is acquired using information dependent acquisition (IDA) with high sensitivity mode selected. The parameters were set as follows: the collision energy (CE) was fixed at 35 V with ± 15 eV; declustering potential (DP), 60 V (+) and −60 V (−); exclude isotopes within 4 Da, candidate ions to monitor per cycle: 10.

(3) Data processing

The raw MS data (wiff.scan files) were converted to MzXML files using ProteoWizard MSConvert before importing into freely available XCMS software. For peak picking, the following parameters were used: centWave m/z = 25 ppm, peakwidth = c (10, 60), prefilter = c (10, 100). For peak grouping, bw = 5, mzwid = 0.025, minfrac = 0.5 were used. CAMERA (Collection of Algorithms of MEtabolite pRofile Annotation) was ued for annotation of isotopes and adducts. In the extracted ion features, only the variables having more than 50% of the nonzero measurement values in at least one group were kept. Compound identification of metabolites was performed by comparing of accuracy m/z value (<25 ppm), and MS/MS spectra with an in-house database established with available authentic standards.

(4) Statistical analysis

After normalized to total peak intensity, the processed data were uploaded into MetaboAnalyst software for further analysis (www.metaboanalyst.ca). Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were performed for both positive and negative models after log transformation and Pareto-scaling. The variable importance in the projection (VIP) value of each variable in the PLS-DA model was calculated to indicate its contribution to the classification. Metabolites with the VIP value >1 was further applied to Student's t-test at univariate level to measure the significance of each metabolite, $p < 0.05$ were considered as statistically significant.

Text S4 Transcriptomics analysis of testis

1. Construction and sequencing of cDNA library for RNA seq

(1) Total RNA extraction

The conventional Trizol method was employed to extract total RNA from testicular tissue. The concentration and purity of the extracted RNA were detected using Nanodrop (the concentration of RNA should greater than 50 ng/μL, the values of OD260/280 were between 1.8

and 2.2). RNA integrity was detected by agarose gel electrophoresis and RIN value was determined by Agilent 2100.

(2) Oligo dT enriched mRNA, reverse transcription, and linker adapter

The magnetic beads with Oligo dT were paired with ployA for A-T bases, and mRNA was isolated from total RNA for analysis of transcriptome information. Under the action of reverse transcriptase, stable double stranded cDNA was synthesized by reverse transcription using mRNA as a template. Add End repair mix to double stranded cDNA to form a flat end and add an A base at the 3 'end to connect the Y-shaped junction.

(3) RNA seq fragment screening, library enrichment, and sequencing

Purify and sort the products connected to the adapter mentioned above, and then perform PCR amplification to obtain the final library. Utilizing QuantiFluor ® Quantitative analysis was conducted using the dsDNA System. Cluster was generated by bridge PCR amplification on cBot and then sequenced using Illumina.

2. RNA seq data quality control and de novo assembly

The specific operation is as follows:

(1) Remove the joint sequence from reads;

(2) Cut off bases with a mass less than 20 and remove reads with a N content ratio exceeding 10%;

(3) Remove the adapter and sequences with a length less than 20bp;

(4) Using Trinity software (https://github.com/trinityrnaseq/trinityrnaseq/wiki) assembles short fragment sequences, including three steps: Inchworm, Chrysalis, and Butterfly.

(5) TransRate (http://hibberdlab.com/transrate/) and CD-HIT (http://weizhongli-lab.org/cd-hit/) were employed to remove the redundant and similar sequences. Using software BUSCO (Benchmarking Universal Single Copy Orthologs, http://busco.ezlab.org) to evaluate the assembly integrity of transcriptome.

3. RNA seq differential expression analysis

Using software RSEM (http://deweylab.github.io/RSEM/) to analyze the genes expression level analysis and locate the number of reads counts in the genome region to calculate the gene expression level. Next, the software edgeR (http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html) or DESeq2 (http://bioconductor.org/packages/stats/bioc/DESeq2/) was used to analyze the differential

expression of genes and identify the differentially expressed genes between samples. FDR<0.05 and | log2FC | ≥ 1 was considered as a differentially expressed gene.

4. RNA seq Functional Enrichment Analysis

Using the KEGG database (http://www.genome.jp/kegg/) to analyze the pathway enrichment of differentially expressed genes (p<0.05 wass considered significant enrichment).Finally,the potential pathways and targets of action were identified.

Table S1 Amino acid composition of SCP.

Amino acid	Absolute content rate (%)
Asp	8.48
Glu	12.92
Ser	3.00
Gly	20.37
His	0.29
Arg	6.22
Thr	3.91
Ala	9.57
Pro	8.60
Tyr	0.88
Val	2.85
Met	0.74
Cys	0.11
Ile	1.40
Leu	2.69
Phe	0.65
Lys	0.95

Table S2 Food intake.

	1st week	2nd week	3rd week	4th week	5th week	6th week
			(g/per mouse/one day)			
Blank+Vehicle	4.77±0.96	4.24±0.54	4.19±0.27	4.03±0.38	4.04±0.25	3.94±0.37
AES+Vechicle	4.2±0.00	3.77±0.18	3.72±0.07	3.92±0.15	3.82±0.10	3.88±0.11
AES+SCPL	4.09±0.27	3.80±0.30	3.55±0.31	3.65±0.01	3.68±0.06	3.58±0.28
AES+SCPH	4.10±0.02	3.93±0.14	3.75±0.07	3.87±0.30	3.76±0.18	3.66±0.10

Data are expressed as mean ± SD (n=8). Compared with the Blank+Vehicle group, * is $p<0.05$, and * is $p<0.01$. Compared with the AES+Vehicle group, # is $p<0.05$, # is $p<0.01$, and unmarked indicates no statistical difference ($p>0.05$).

Table S3 Organ index of mice.

	Organ indexes（%）					
	Liver	Kidney	Testis	Seminal vesicle	Epididymis	Penis
Blank+Vehicle	5.29±0.38	1.50±0.06	0.67±0.09	0.74±0.12	0.27±0.07	0.15±0.01
AES+Vehicle	4.99±0.25	1.47±0.18	0.73±0.04	0.48±0.11**	0.28±0.07	0.11±0.04*
AES+SCPL	5.22±0.35	1.43±0.09	0.77±0.07*	0.65±0.12	0.31±0.08	0.16±0.02#
AES+SCPH	5.15±0.52	1.45±0.16	0.77±0.08	0.63±0.16	0.32±0.09	0.16±0.02#

Data are expressed as mean ± SD (n=8). Compared with the Blank+Vehicle group, * is $p<0.05$, and * is $p<0.01$. Compared with the AES+Vehicle group, # is $p<0.05$, # is $p<0.01$, and unmarked indicates no statistical difference ($p>0.05$).

Table S4 Serum biomarkers comparison at negative iron model (Top25).

No.	Metabolite	m/z	Fold change1	Trend1 (TrnM)	VIP1	P-Value1	Fold change2	Trend2 (TrnM)	VIP2	P-Value2
1	L-Tryptophan	203.08	2.35	+	5.57	0.00000	0.47	-	5.20	0.00000
2	Pentadecanoic Acid	241.22	1.57	+	2.42	0.00000	0.74	-	1.93	0.00052
3	Indole	116.05	2.14	+	1.19	0.00000	0.51	-	1.12	0.00000
4	L-Aspartate	132.03	1.65	+	1.22	0.00001	0.61	-	1.26	0.00002
5	Uridine	243.06	2.34	+	7.45	0.00006	0.53	-	6.47	0.00116
6	9,10-DiHOME	313.24	2.18	+	3.22	0.00010	0.59	-	2.56	0.00408
7	Sphingosine-1-phosphate	378.24	0.63	-	1.01	0.00014	1.83	+	1.11	0.00001
8	Succinate	117.02	1.47	+	1.09	0.00018	0.71	-	1.13	0.00053
9	Deoxycholic acid	391.28	1.95	+	2.12	0.00065	0.68	-	1.63	0.01269
10	Myristoleic acid	225.18	0.40	-	2.41	0.00112	3.36	+	2.83	0.00666
11	Genistein	269.05	2.28	+	1.56	0.00114	0.38	-	1.82	0.00095
12	L-Malic acid	133.01	1.76	+	1.20	0.00119	0.61	-	1.20	0.00482
13	3-Methoxy-4-Hydroxyphenylglycol Sulfate	263.02	1.67	+	1.25	0.00147	0.66	-	1.09	0.00384
14	Dihomo-gamma-Linolenic	305.25	2.06	+	2.03	0.00232	0.55	-	1.99	0.05342
15	DL-lactate	89.02	1.26	+	3.09	0.00236	0.79	-	4.68	0.00144
16	Thymine	125.03	1.52	+	1.03	0.00707	0.52	-	1.13	0.00105
17	Xanthosine	283.07	2.49	+	2.10	0.01115	0.37	-	2.20	0.00665
18	Uric acid	167.02	2.07	+	2.21	0.01250	0.44	-	2.31	0.00360
19	Daidzein	253.05	1.78	+	1.13	0.01616	0.49	-	1.40	0.00624
20	3R-hydroxy-butanoic acid	103.04	0.36	-	1.91	0.01731	2.76	+	2.01	0.00063
21	3-Hydroxydodecanoic acid	215.16	0.65	-	1.21	0.01946	1.65	+	1.21	0.03017
22	Alpha-D-Glucose	179.05	2.65	+	1.36	0.02234	0.43	-	1.43	0.03743

23	L-Ascorbic acid	197.00	1.30	+	3.50	0.04731	0.36	-	11.04	0.00076
24	L-Glutamine	145.06	1.34	+	3.65	0.64269	1.08	+	3.71	0.025
25	9R,10S-EpOME	295.23	1.77	+	4.89	0.00001	0.68	-	3.88	0.00098

Note: Trend1 refers to the AES+Vehicle group being up-regulated as "+" and down-regulated as "-" compared to the blank+Vehicle group. Trend2 represents the upregulation of the AES+SCPH group to "+" and downregulation to "-" compared to the AES+Vehicle group.

Table S5 Serum biomarkers comparison at positive iron model (Top25).

No.	Metabolite	m/z	Fold change1	Trend1 (TrnM)	VIP1	P-Value1	Fold change2	Trend2 (TrnM)	VIP2	P-Value2
1	3-Indoleacetonitrile	174.10	1.54	+	1.13	0.00000	0.60	-	1.24	0.00000
2	L-Tryptophan	205.10	1.89	+	1.81	0.00001	0.45	-	2.03	0.00000
3	Thymine	127.05	1.77	+	1.93	0.00003	0.91	-	0.78	0.23691
4	Creatinine	114.07	1.27	+	1.96	0.00004	0.82	-	1.77	0.00003
5	D-Proline	116.07	1.45	+	1.33	0.00006	0.70	-	1.32	0.00013
6	Tyramine	120.08	1.29	+	3.49	0.00008	0.76	-	3.58	0.00018
7	20-Hydroxyarachidonic acid	303.23	1.98	+	1.82	0.00025	0.65	-	1.46	0.00231
8	L-Leucine	132.10	1.26	+	1.45	0.00025	0.75	-	1.69	0.00001
9	1-Palmitoylglycerol	313.27	1.77	+	1.52	0.00027	0.51	-	1.67	0.00020
10	1-Palmitoyllysophosphatidylcholine	538.39	1.33	+	4.81	0.00044	0.77	-	4.42	0.00413
11	Urea	61.04	1.16	+	1.83	0.00145	0.84	-	2.05	0.00036
12	1-Oleoyl-sn-glycero-3-phosphocholine	544.35	0.73	-	8.03	0.00164	1.41	+	9.06	0.00626
13	2-Methylbutyroylcarnitine	246.17	1.33	+	3.47	0.00337	0.71	-	3.83	0.00068
14	Daidzein	255.07	2.73	+	3.65	0.00493	0.27	-	4.08	0.00236

15	1-Stearoyl-2-oleoyl-sn-glycerol 3-phosphocholine (SOPC)	810.60	0.76	-	4.40	0.00618	1.23	+	4.79	0.02392
16	Indoleacetic acid	158.06	1.80	+	2.76	0.00661	0.53	-	3.16	0.00033
17	Genistein	271.06	1.74	+	1.20	0.00675	0.38	-	1.59	0.00069
18	Choline	104.11	1.11	+	1.38	0.00677	0.91	-	1.16	0.04749
19	L-Glutamine	169.06	0.88	-	1.18	0.01500	1.09	+	1.14	0.102512
20	Glycitein	285.08	2.74	+	1.64	0.01693	0.43	-	1.56	0.03886
21	1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	454.28	0.85	-	1.87	0.01994	1.09	+	1.23	0.21528
22	Acetylcarnitine	204.12	0.82	-	7.85	0.03353	1.33	+	10.62	0.00133
23	L-Carnitine	162.11	0.81	-	2.76	0.03510	1.19	+	2.90	0.00301
24	1-Methylhistidine	170.09	1.18	+	1.73	0.04675	0.79	-	2.14	0.00914
25	1-Stearoyl-2-arachidonoyl-sn-glycerol	810.60129	0.76	-	4.40	0.00618	1.28	+	4.28	0.01095

Note: Trend1 refers to the AES+Vehicle group being up-regulated as "+" and down-regulated as "-" compared to the blank+Vehicle group. Trend2 represents the upregulation of the AES+SCPH group to "+" and downregulation to "-" compared to the AES+Vehicle group.

Fig. S1. The acute exhausted swimming (AES) and copulatory test

Fig. S2. Differential serum metabolites heat map in positive mode (A) and negative mode (B).

Fig. S3. Serum metabolites of AES mice.

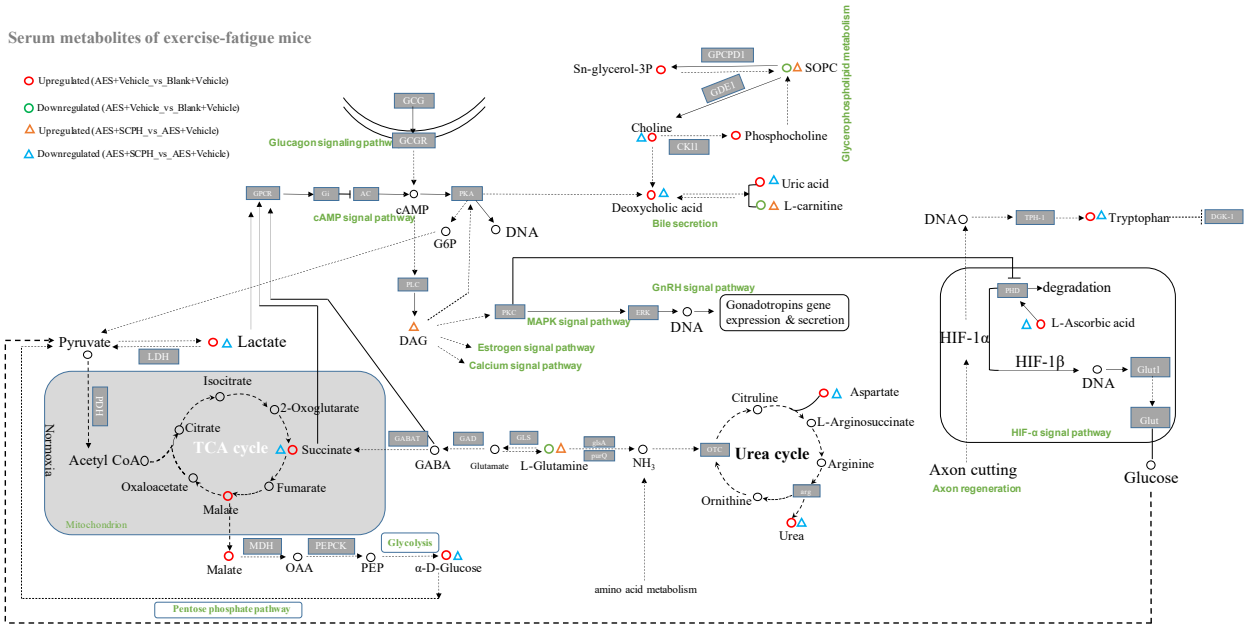


Fig. S4. Testicular transcriptome cluster analysis of differentially expressed common genes.

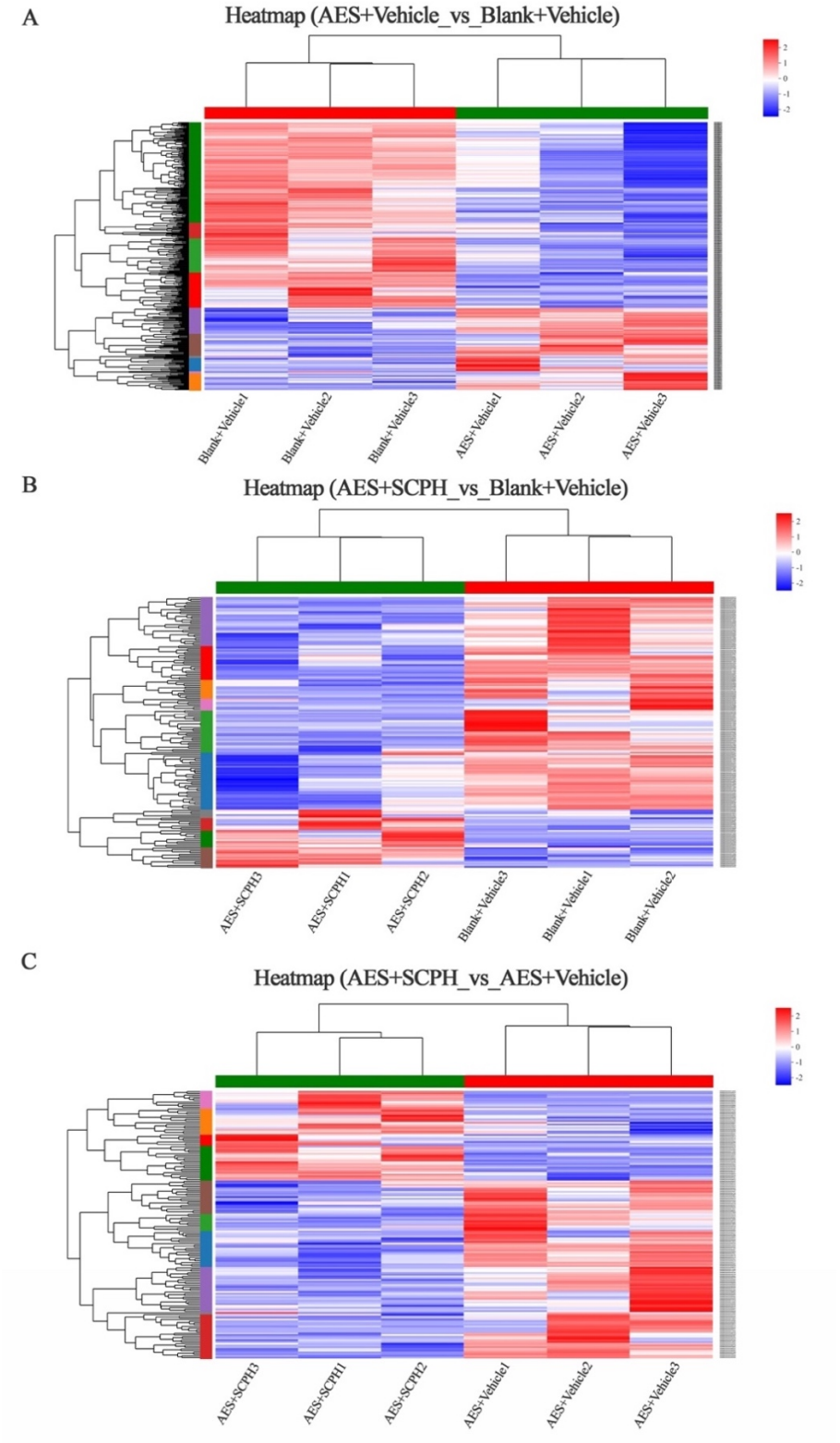
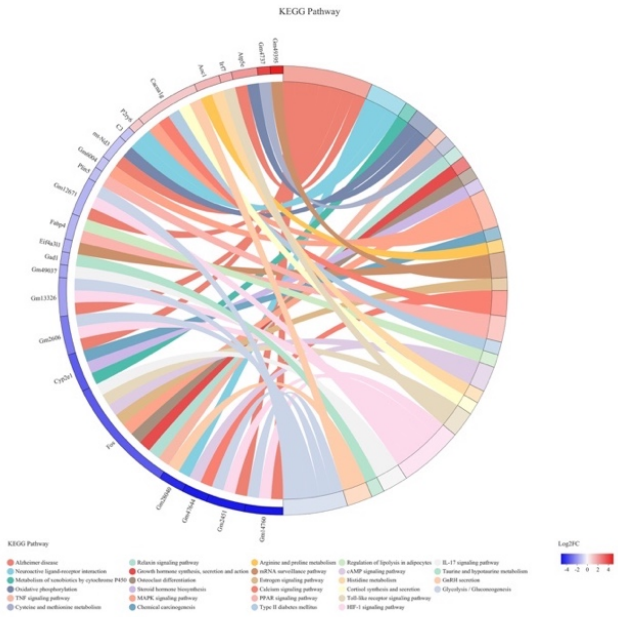


Fig. S5. Testicular transcriptome chord diagram of KEGG pathway enrichment for AES+SCPH_vs_AES+Vehicle gene set.



gene name	KEGG Pathway	log2fc
Gm13326	Alzheimer disease;HIF-1 signaling pathway;Glycolysis/Gluconeogenesis	-1.85
Gad1	Taurine and hypotaurine metabolism	-1.59
Cacna1g	MAPK signaling pathway;Calcium signaling pathway;Type II diabetes mellitus;Cortisol synthesis and secretion;GnRH secretion	1.06
Gm2606	Alzheimer disease;HIF-1 signaling pathway;Glycolysis/Gluconeogenesis;	-2.63
Eif4a3l1	mRNA surveillance pathway	-1.51
Gm14760	Alzheimer disease;HIF-1 signaling pathway;Glycolysis/Gluconeogenesis	-4.81
Irf7	Toll-like receptor signaling pathway	1.63
Gm2451	Alzheimer disease;HIF-1 signaling pathway;Glycolysis/Gluconeogenesis	-4.72
Gm4737	Cysteine and methionine metabolism	4.00
Gm6004	MAPK signaling pathway	-1.10
Atp5e	Alzheimer disease;Oxidative phosphorylation	2.15
Plin5	PPAR signaling pathway	-1.18
P2ry6	Neuroactive ligand-receptor interaction	1.05
Fabp4	PPAR signaling pathway;Regulation of lipolysis in adipocytes	-1.41
C3	Neuroactive ligand-receptor interaction	-1.04
Fos	TNF signaling pathway;Relaxin signaling pathway;Growth hormone synthesis, secretion and action;Osteoclast differentiation;MAPK signaling pathway;Estrogen signaling pathway;cAMP signaling pathway;Toll-like receptor signaling pathway;IL-17 signaling pathway	-3.33
Gm28040	Neuroactive ligand-receptor interaction;GnRH secretion	-4.40
Gm12671	Alzheimer disease;HIF-1 signaling pathway;Glycolysis/Gluconeogenesis	-1.40
mt-Nd3	Alzheimer disease;Oxidative phosphorylation	-1.04
Cyp2e1	Metabolism of xenobiotics by cytochrome P450;Steroid hormone biosynthesis;Chemical carcinogenesis	-3.29
Gm47644	Calcium signaling pathway;cAMP signaling pathway	-4.69
Aoc1	Arginine and proline metabolism;Histidine metabolism	1.46
Gm49395	mRNA surveillance pathway;IL-17 signaling pathway	4.86

References

1. Z. W. Liu, N. Jiang, X. Tao, X. P. Wang, X. M. Liu and S. Y. Xiao, Assessment of Sexual Behavior of Male Mice, *J Vis Exp*, 2020, DOI: 10.3791/60154.