**Supplemental Information**

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**Supplementary Figure 1.** The variation of cholesterol level in the liver of Chinese soft-shelled turtle between hibernation and non-hibernation period.

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**Supplementary Figure 2.** Periodic Acid-Schiff (PAS) staining of the Chinese soft-shelled turtle liver tissue in hibernation and non-hibernation period. A, C: PAS staining of liver tissue in non-hibernation period; B, D: PAS staining of liver tissue in hibernation period.

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**Supplementary Figure 3.** The statistics of differentially expressed genes (DEGs) between CK and T libraries. A: Volcano plot. B: Differentially expressed genes statistics.

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**Supplementary Figure 4.** The histogram of Gene Ontology (GO) enrichment classification.

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**Supplementary Figure 5.** Top 20 of the most enriched GO terms among DEGs in the liver of Chinese soft-shelled turtle.



**Supplementary Figure 6.** Expression authentication of selected DEGs in the liver of Chinese soft-shelled turtle. A: Heat map of gene expression mode of DEGs in the liver of Chinese soft-shelled turtle. B: Relative mRNA levels of DEGs by RT-qPCR (blue bar) were in contrast with transcript abundances of RNA-Seq (green bar).

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**Supplementary Figure 7.** Western blotting verification of protein expression in metabolic pathways. A, B: The expression level of proteins related to carbohydrate metabolism. C, D: The expression level of proteins involved in lipid and fatty acid metabolism.

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**Supplementary Figure 8.** Western blotting verification of protein expression in lipolysis and autophagy. A, B: The expression level of proteins related to lipolysis. C, D, E: The expression level of proteins involved in autophagy.



**Supplementary Figure 9.** Top 20 of the most enriched KEGG pathway among DEGs in the liver of Chinese soft-shelled turtle.

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**Supplementary Figure 10.** 3MA inhibition blocks autophagy in cultured hepatocytes of Chinese soft-shelled turtle. A: The expression level of autophagy related proteins (LC3 and p62); B, C: Statistics of the western blotting results.

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**Supplementary Figure 11.** Expression of autophagy related protein in primary hepatocyte from Chinese soft-shelled turtle (*Pelodiscus sinensis*) after DEUP inhibition. A: Expression of autophagy related protein (LC3 and p62) by Western blotting; B, C: Statistics of the western blotting results.

**Supplement Table 1** Primers of DEGs for RT-qPCR analysis in The Chinese soft-shelled turtle.

|  |  |  |
| --- | --- | --- |
| **Gene name** | **Sense primer (5'-3')** | **Antisense primer (5'-3')** |
| *G6PC* | AGCATTTGAGCACATTCACG | AAATAGGATGCCCAGGTTCC |
| *GYS2* | TCATCCGAGGCCATTTCTAC | GCTTGGCCTTTCAGTGTTTC |
| *HK1* | GGGAGAAATCGTTCGCAATA | TGCTCGTACCTGAAGCAATG |
| *PFKFB3* | ACGAGCATGTGGCTTCTTCT | AGTCTGGTCTGCCCTAAGCA |
| *ACADM* | GGCCTTGGCTCTTTTGAAGC | GCTCCAGGTTCTGTTACGCA |
| *ACSL1* | ACCAATCTGACAAGGCCAAG | CTGTGCTCTCTTCCCACCTC |
| *ACACA* | TAGTGTCCAGCATCGTCAGC | GAATGAGTTGTCCAGCAGCA |
| *GPAT4* | CCATCCTCATCTTCCCAGAA | GTCACCATCCCGTACTTGCT |
| *AGPAT3* | AAGCTGCAAACTGGCTTCAT | AAGGAAGCTGCTCCAACAAA |
| *PNPLA2* | AACCTTCGCAACCTCTACCG | GCGGTGAAGGAGACCATTCT |
| *PPARA* | ACAGGTGAACAGGATGTGGAA | TCTCTGCCATACACAGCGTT |
| *DGAT2* | AAGCATGAATGGGAAGAGGC | GTTACAGTGCAAGCAACACCC |
| *ME1* | ACGGCATGTGGAGGAATGAA | AGGCTTGACCCCTGACTCTT |
| *G0S2* | TTCTTCGGGGTGGTCATTAG | CTTTGCTTTTCCCCACAGTC |
| *PIK3R3* | AACCTTCGCAACCTCTACCG | GCGGTGAAGGAGACCATTCT |
| *MAP1LC3B* | CAGCCATCCAGGCATTGAGA | CTTGCTAAGATCGGACGGGG |
| *ATG16L1* | ACAGGTGAACAGGATGTGGAA | TCTCTGCCATACACAGCGTT |
| *PIK3R1* | GCTACTCTCGTTGCAGTGGT | GGATACGTTGCCCTGAGAGG |
| *NBR1* | AAGCATGAATGGGAAGAGGC | GTTACAGTGCAAGCAACACCC |
| *β-actin* | AGACCCGACAGACTACCTCA | CACCTGACCATCAGGCAACT |

**Supplement Table 2** Statistics of sequenced reads and genes.

|  |  |  |
| --- | --- | --- |
| **Sample Names** | **CK** | **T** |
| Clean Reads | 66661832 | 46619602 |
| Total Mapped (%) | 56309200 (84.47%) | 38464271 (82.51%) |
| Multiple Mapped (%) | 2675382 (4.01%) | 2017007 (4.33%) |
| Unique Mapped (%) | 53633818 (80.46%) | 36447264 (78.18%) |
| Sequenced Refer Genes (%) | 17061 (69.65%) | 16096 (65.71%) |
| Sequenced Novel Genes | 6116 | 6053 |
| Sequenced Total Genes | 23177 | 22149 |

**Supplement Table 3** Differentially Expressed Genes (DEG) involved in carbohydrate metabolism.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene ID** | **CK-fpkm** | **T-fpkm** | **Log2(T/CK)** | **P-value** | **Gene Symbol** | **Description** |
| **UP-regulated genes** |  |  |  |  |  |  |
| LOC102443987 | 72.42 | 147.27 | 1.024003 | *5.18E-274* | *G6PC* |  |
| LOC106731579 | 440.25 | 544.52 | 0.306662 | *1.71E-43* | *GTP* |  |
| LOC102449457 | 20.74 | 24.03 | 0.212421 | *2.61E-08* | *GYS2* |  |
| LOC102461748 | 3.92 | 6.77 | 0.788302 | *1.59E-42* | *GSK3B* |  |
| LOC102463406 | 25.35 | 33.63 | 0.407763 | *1.44E-09* | *GSK3A* |  |
| **DOWN-regulated genes** |  |  |  |  |  |  |
| MSTRG.16977 | 15.29 | 0.62 | -4.62418 | *1.278E-180* | *LDHA* |  |
| MSTRG.10155 | 259.02 | 115.96 | -1.15944 | *1.05401988* | *GAPDH* |  |
| MSTRG.2417 | 32.55 | 11.82 | -1.46143 | *2.71E-115* | *PHDA1* |  |
| LOC102462504 | 34.14 | 3.23 | -3.40186 |  | *DLD* |  |
| LOC102451343 | 0.93 | 0.26 | -1.83872 | *2.37E-11* | *HK1* |  |
| MSTRG.1341 | 2.6 | 0.46 | -2.49881 | *2.77E-22* | *PFKP* |  |
| LOC102449400 | 148.76 | 67.01 | -1.15054 | *9.94E-187* | *ALDOA* |  |
| LOC102462309 | 13.34 | 6.3 | -1.08233 | *1.01E-39* | *GALM* |  |
| LOC102447629 | 5.64 | 2.11 | -1.41845 | *2.50E-26* | *BPGM* |  |
| LOC102458605 | 2.08 | 0.94 | -1.14585 | *1.21E-06* | *MINPP1* |  |
| MSTRG.8754 | 42.28 | 12.62 | -1.74426 | *9.09E-300* | *ADPGK* |  |
| MSTRG.11459 | 4.76 | 1.67 | -1.51111 | *9.62E-12* | *GCK* |  |
| LOC102444798 | 8.16 | 4.87 | -0.74465 | *2.81E-24* | *PFKFB3* |  |

**Supplement Table 4** Differentially Expressed Genes (DEG) involved in lipid and fatty acid metabolism.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene ID** | **CK-fpkm** | **T-fpkm** | **Log2(T/CK)** | **P-value** | **Gene Symbol** | **Description** |
| **UP-regulated genes** |  |  |  |  |  |  |
| MSTRG.12079 | 7.49 | 12.41 | 0.728465 | *2.75E-38* | *ACACB* |  |
| LOC102461261 | 45.63 | 121.29 | 1.410406 |  | *HADH* |  |
| LOC102462849 | 70.53 | 89.27 | 0.339938 | *1.52E-24* | *ACADM* |  |
| LOC102444046 | 6.19 | 26.46 | 2.095802 | *2.40E-90* | *ECHS1* |  |
| LOC102454353 | 70.96 | 61.04 | -0.21725 | *4.82E-12* | *ELOVL5* |  |
| LOC102453641 | 1.82 | 46.83 | 4.685423 |  | *ECI2* |  |
| LOC102443833 | 1.36 | 2.81 | 1.046963 | *3.41E-15* | *PNPLA2* |  |
| LOC102444584 | 261.55 | 462.61 | 0.82271 | *7.08E-259* | *ALDH2* |  |
| LOC102459845 | 2.75 | 3.51 | 0.352039 | *0.0095176* | *GK* |  |
| LOC102454165 | 2.05 | 2.72 | 0.407983 | *0.0193234* | *DGK* |  |
| LOC102456906 | 76.06 | 158.63 | 1.060456 |  | *LPL* |  |
| MSTRG.18683 | 28.72 | 71.94 | 1.324738 |  | *ABCB11* |  |
| LOC102444975 | 13.05 | 25.82 | 0.984439 | *2.16E-137* | *NPC1* |  |
| LOC102450102 | 0.22 | 0.71 | 1.690316 | *9.35E-09* | *VLDLR* |  |
| MSTRG.7430 | 4.94 | 6.98 | 0.498716 | *1.51E-07* | *LDLRAP1* |  |
| LOC102453310 | 1.88 | 2.22 | 0.239827 | *0.2147972* | *CETP* |  |
| LOC102453323 | 37.56 | 54.51 | 0.537324 | *1.16E-41* | *STARD3* |  |
| MSTRG.15825 | 454.74 | 520.52 | 0.194912 | *7.61E-21* | *APOB* |  |
| LOC102452004 | 60.67 | 123.9 | 1.030121 | *3.96E-195* | *UQCRC1* |  |
| LOC102449220 | 96.2 | 116.98 | 0.282153 | *2.31E-07* | *UQCRQ* |  |
| LOC102460622 | 61.59 | 85.41 | 0.471709 | *5.10E-60* | *ATP1A1* |  |
| LOC102463291 | 2.06 | 2.28 | 0.146389 | *0.3972403* | *ACOX1* |  |
| LOC102447272 | 3.7 | 7.59 | 1.036575 | *3.37E-16* | *COX15* |  |
| MSTRG.11720 | 3.42 | 18.19 | 2.411077 | *1.23E-177* | *COX5A* |  |
| LOC102444236 | 216.87 | 294.87 | 0.443249 | *2.52E-26* | *COX6A* |  |
| LOC102462699 | 31.74 | 54.52 | 0.780483 | *1.37E-27* | *COX7A2* |  |
| LOC112546923 | 272.51 | 339.85 | 0.318589 | *1.56E-27* | *COX4I1* |  |
| LOC102451069 | 1.29 | 15.37 | 3.574674 | *3.17E-139* | *ATP6AP1* |  |
| LOC102447058 | 15.02 | 18.54 | 0.303756 | *3.08E-06* | *VDAC1* |  |
| LOC102459419 | 26.7 | 43.43 | 0.701852 | *1.82E-20* | *SDHAF2* |  |
| LOC102450342 | 64.23 | 68.17 | 0.08589 | *0.152633* | *NDUFB10* |  |
| **DOWN-regulated genes** |  |  |  |  |  |  |
| LOC102462443 | 542.57 | 356.64 | -0.60534 | *1.66E-164* | *ACSL1* |  |
| LOC102453486 | 3.04 | 1.1 | -1.46657 | *5.62E-62* | *ACACA* |  |
| LOC102447625 | 2.11 | 1.96 | -0.10639 | *0.5337028* | *OXSM* |  |
| LOC102462953 | 18.95 | 7.29 | -1.37821 | *2.67E-62* | *MECR* |  |
| LOC112544543 | 35.45 | 9.09 | -1.96343 | *3.30E-93* | *HSD17B12* |  |
| LOC102451471 | 20.13 | 4.83 | -2.05925 | *7.01E-167* | *GPAT4* |  |
| LOC102443697 | 1.26 | 1.00 | -0.33342 | *0.1176291* | *GPAT3* |  |
| LOC102452592 | 7.08 | 2.24 | -1.66025 | *5.06E-22* | *PLPP3* |  |
| LOC102460931 | 4.24 | 1.92 | -1.14296 | *1.40E-13* | *PLPP2* |  |
| LOC102449336 | 38.1 | 14.2 | -1.4239 | *2.36E-113* | *DGAT2* |  |
| LOC102448805 | 10.07 | 8.9 | -0.17819 | *0.130482* | *AGPAT2* |  |
| LOC102446025 | 2.8 | 1.67 | -0.74558 | *8.56E-10* | *AGPAT3* |  |
| LOC102445720 | 7.81 | 5.86 | -0.41442 | *4.51E-05* | *ACSF3* |  |
| LOC102452290 | 2.08 | 0.67 | -1.63435 | *1.55E-14* | *ABCG5* |  |

**Supplement Table 5** Differentially Expressed Genes (DEG) involved in lipolysis.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene ID** | **CK-fpkm** | **T-fpkm** | **Log2(T/CK)** | **P-value** | **Gene Symbol** | **Description** |
| **UP-regulated genes** |  |  |  |  |  |  |
| LOC102443833 | 1.36 | 2.81 | 1.046963 | *3.41E-15* | *PNPLA2* |  |
| MSTRG.15825 | 454.74 | 520.52 | 0.194912 | *7.61E-21* | *APOB* |  |
| LOC102451519 | 0.44 | 1.13 | 1.360747 | *2.03E-11* | *PPARA* |  |
| LOC102463291 | 2.06 | 2.28 | 0.146389 | *0.3972403* | *ACOX1* |  |
| LOC102459501 | 253.33 | 483.42 | 0.932259 | *1.66E-204* | *FABP1* |  |
| LOC102463898 | 23.13 | 25.11 | 0.118497 | *0.068172* | *ABHD5* |  |
| LOC102455043 | 1.61 | 2.36 | 0.551726 | *3.38E-06* | *PLIN1* |  |
| LOC102459979 | 31.33 | 99.3 | 1.664249 |  | *PLIN3* |  |
| MSTRG.14452 | 63.81 | 330.73 | 2.373799 |  | *PLIN2* |  |
| **DOWN-regulated genes** |  |  |  |  |  |  |
| LOC102449336 | 38.1 | 14.2 | -1.4239 | *2.36E-113* | *DGAT2* |  |
| LOC102453881 | 1.02 | 0.42 | -1.28011 | *5.64E-08* | *ME1* |  |
| LOC102458904 | 355.14 | 132.86 | -1.41848 |  | *G0S2* |  |

**Supplement Table 6** Differentially Expressed Genes (DEG) involved in autophagy.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene ID** | **CK-fpkm** | **T-fpkm** | **Log2(T/CK)** | **P-value** | **Gene Symbol** | **Description** |
| **UP-regulated genes** |  |  |  |  |  |  |
| LOC102457516 | 1.19 | 2.89 | 1.280108 | *6.35E-14* | *PIK3R3* |  |
| LOC102458748 | 0.84 | 10.93 | 3.70176 | *2.34E-252* | *RAF1* |  |
| LOC102449711 | 0.55 | 4.38 | 2.993427 | *1.36E-96* | *DEPTOR* |  |
| LOC102443852 | 4.2 | 8.57 | 1.028906 | *2.87E-47* | *ULK1* |  |
| LOC102460163 | 116.92 | 131.34 | 0.167785 | *8.19E-09* | *MAP1LC3B* |  |
| LOC102462041 | 7.16 | 10.26 | 0.518999 | *2.59E-16* | *ATG16L1* |  |
| LOC102454205 | 4.32 | 5.58 | 0.369234 | *3.14E-07* | *PIK3R4* | *p150* |
| LOC102459596 | 0.26 | 0.43 | 0.725825 | *0.0049316* | *PRKAA2* |  |
| **DOWN-regulated genes** |  |  |  |  |  |  |
| MSTRG.12368 | 5.59 | 2.57 | -1.12108 | *4.47E-40* | *PIK3CD* | *p110δ* |
| LOC102449322 | 0.61 | 0.23 | -1.40718 | *0.000915* | *VMP7* |  |
| LOC102449585 | 14.79 | 11.86 | -0.31852 | *1.24E-12* | *PIK3R1* | *p85α* |
| LOC102462122 | 0.55 | 0.21 | -1.38904 | *0.00045* | *PIK3R2* | *p85β* |
| LOC102460136 | 4.29 | 4.27 | -0.00674 | *0.911371* | *PIK3CG* | *p110γ* |
| LOC102462482 | 0.36 | 0.24 | -0.58496 | *0.033343* | *PIK3CA* | *p110α* |
| LOC102453850 | 7.27 | 0.34 | -4.41835 | *5.49E-267* | *AKT1* |  |
| LOC102448379 | 1.98 | 1.62 | -0.28951 | *0.0827794* | *AKT3* |  |
| LOC102451030 | 3.31 | 2.85 | -0.21587 | *0.0016525* | *MTOR* |  |
| LOC102445167 | 1.69 | 0.65 | -1.37851 | *3.23E-21* | *NBR1* |  |