

Supplementary materials

**Figure S1. Statistics of the quantitative proteomics data of different samples.**

(A) The distribution of peptide lengths in all samples. (B) The distribution of peptide coverage in all samples. (C) Numbers of proteins with different masses in all samples. (D) Principal coordinates analysis of four types of individuals. PC: principal coordinate.

**Figure S2. GO and KEGG enrichment analysis of labeled proteins.**

(A) GO enrichment analysis of labeled proteins. (B) KEGG enrichment analysis of labeled proteins.

**Figure S3. GO and KEGG enrichment analysis and subcellular location of abundant proteins in two comparison groups.**

(A) GO enrichment analysis of differential proteins between male and pseudo-female. (B) The subcellular location of differential proteins between male and females. (C) GO enrichment analysis of differential proteins between female and pseudo-male. (D) The subcellular location of differential proteins between female and pseudo-male.

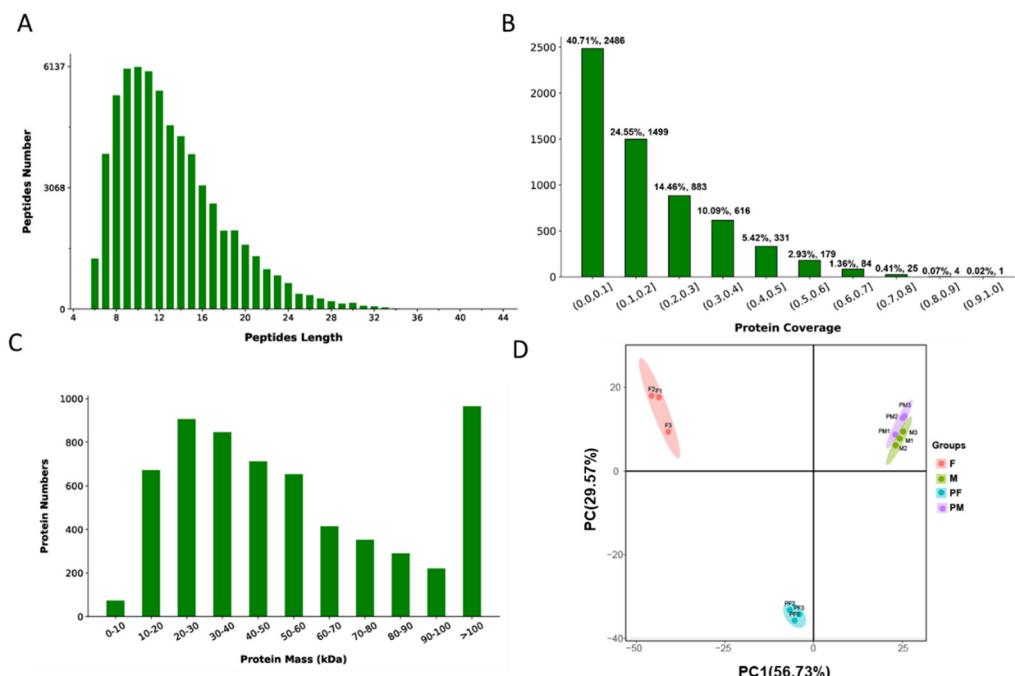
**Figure S4. Cluster analysis on the differentially expressed proteins among four types of Chinese soft-shelled turtles.**

**Supplementary Table S1.** The primers used in this study.

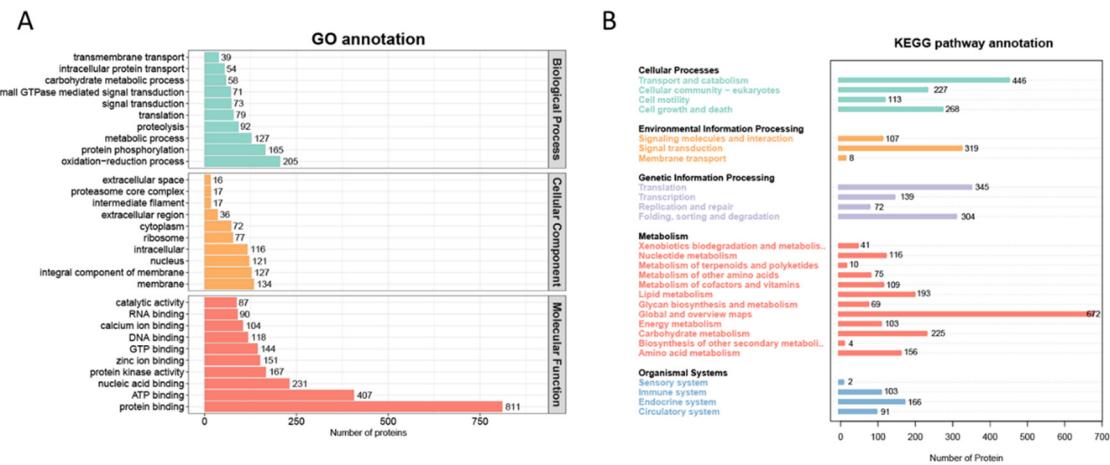
**Supplementary Table S2.** Top 30 differentially expressed proteins between male and pseudo-female.

**Supplementary Table S3.** Top 30 differentially expressed proteins between female and pseudo-male.

**Figure S1**



**Figure S2**



**Figure S3**

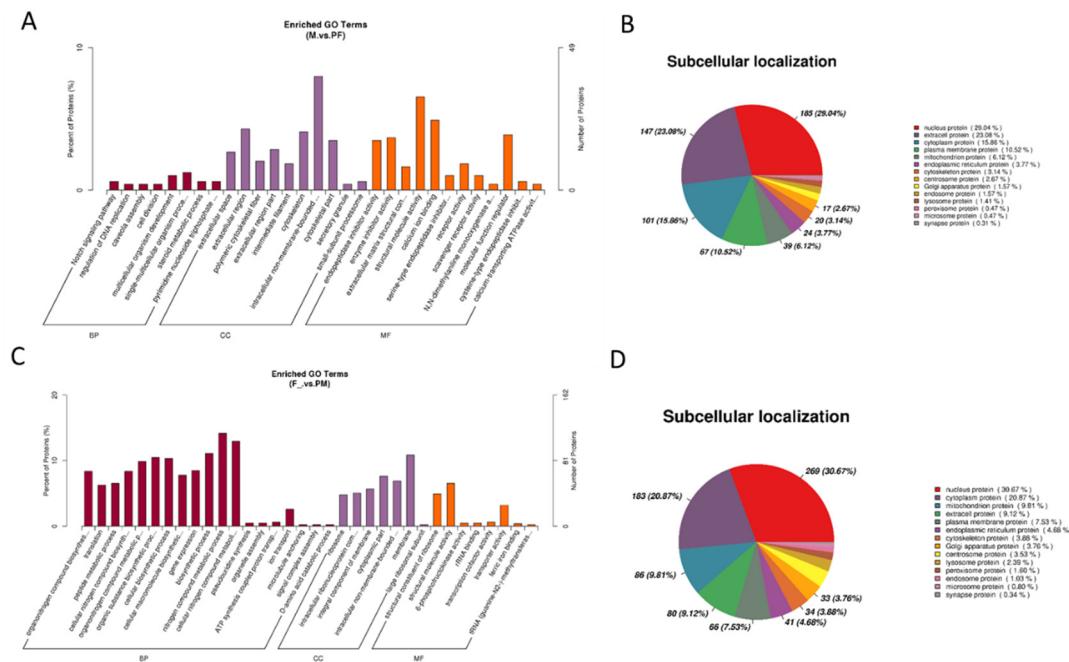
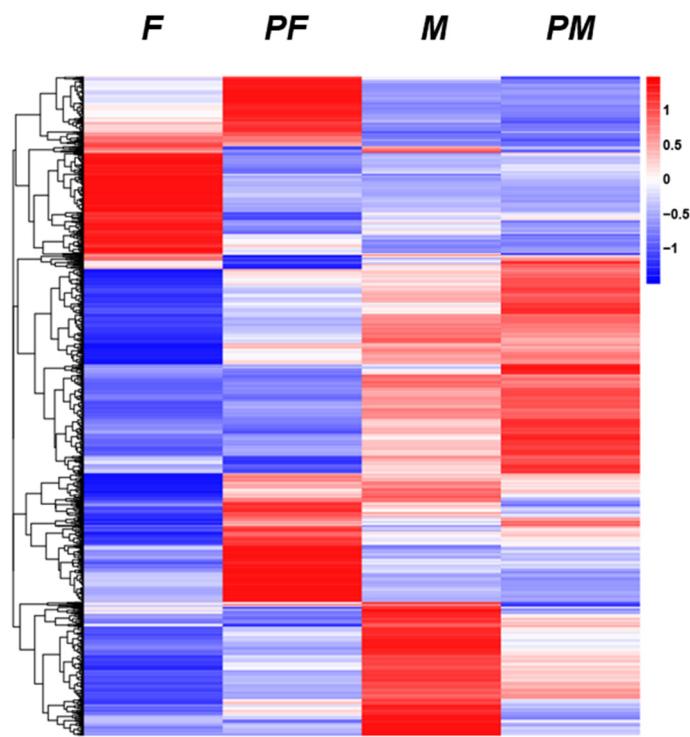


Figure S4



**Supplement Table S1**

| <b>Primers</b> |         | <b>Sequence (5'-3')</b>    |
|----------------|---------|----------------------------|
| <i>K7FKG1</i>  | Forward | GAACAACTTCCCTGCCTGCCTAC    |
|                | Reverse | GCCCATCTTGATGACGCTGATA     |
| <i>K7GIQ2</i>  | Forward | GTCCCAGAAGGCCTCCAGTGG      |
|                | Reverse | CCTCCCGTCCCAGGAACTCAC      |
| <i>COL4A6</i>  | Forward | CCAGCATCCAGGTGCAGATCT      |
|                | Reverse | GTGCCTTGTAACAGCCGAGT       |
| <i>K7F2U2</i>  | Forward | CTACAAATACACCCAGGAGCACC    |
|                | Reverse | AGCAGAGCCCACAGAACAGGA      |
| <i>K7FF80</i>  | Forward | TCATCCCGCTCCTCCTTTTC       |
|                | Reverse | GCTCCTGTTGCAGGCAACGT       |
| <i>RPL28</i>   | Forward | CGACGGCAAGGGCATTGTGGT      |
|                | Reverse | ACGCAGGCTGTTGAGGGTGGC      |
| <i>SRSF3</i>   | Forward | GAACAACTTCCCTGCCTGCCTAC    |
|                | Reverse | GCCCATCTTGATGACGCTGATA     |
| <i>SNRNP40</i> | Forward | GTCCTGCCTACCAACGGCTCA      |
|                | Reverse | GCATTGCCTGTTCTACAGACCC     |
| <i>HNRNPK</i>  | Forward | GGGAAAGCCGACGGAAAGAG       |
|                | Reverse | GGTGCAGCAAAGCTATGATGACAC   |
| 4085           | Forward | GTTTGAAGTGCTGCTGGGAAG      |
|                | Reverse | TTCCCCGTATAAAGCCAGGG       |
| <i>COI</i>     | Forward | CAACCAACCACAAGACATTGGCAC   |
|                | Reverse | ACCTCAGGGTGTCCGAARAATCARAA |

**Supplement Table S2**

| Protein | M1     | M2     | M3     | PF1     | PF2     | PF3     | Up | Down |
|---------|--------|--------|--------|---------|---------|---------|----|------|
| K7FE08  | 11.4   | 9.9    | 11.8   | 120.5   | 114     | 102.5   | *  | --   |
| K7F5N5  | 93     | 68.3   | 52.4   | 525.5   | 523.5   | 569.8   | *  | --   |
| K7FDA4  | 85.9   | 122.5  | 59.5   | 670.3   | 640.4   | 680.3   | *  | --   |
| K7FJB7  | 89.4   | 83.9   | 94.3   | 673.3   | 607.7   | 624.4   | *  | --   |
| TSPAN1  | 21.4   | 22.8   | 16     | 139.7   | 140.3   | 136.9   | *  | --   |
| K7FTP9  | 96.8   | 113.4  | 79.1   | 630.7   | 648.7   | 643.9   | *  | --   |
| ZP1     | 377.8  | 382.7  | 340.7  | 2336.1  | 2526.1  | 2393    | *  | --   |
| PATL2   | 927.7  | 940.3  | 786.5  | 5466.7  | 5773.5  | 5737.2  | *  | --   |
| MYL9    | 86.7   | 134.3  | 86.8   | 631.2   | 649     | 639.4   | *  | --   |
| K7EW41  | 606.2  | 664.5  | 533.2  | 3640.7  | 3320.7  | 3823    | *  | --   |
| RBP7    | 688.5  | 685.4  | 580.9  | 3802.3  | 4073.2  | 3558.3  | *  | --   |
| LSM14B  | 323    | 287    | 265.9  | 1607.3  | 1765.6  | 1613.5  | *  | --   |
| HPCAL4  | 763.1  | 729.6  | 658.5  | 3691    | 4075.3  | 4161.2  | *  | --   |
| COL4A4  | 138.4  | 132.6  | 105.2  | 672.9   | 692.9   | 658.9   | *  | --   |
| ACSL5   | 4038.8 | 4049.7 | 3492.8 | 18486.3 | 19827.2 | 20903.3 | *  | --   |
| K7FSY5  | 840.5  | 835.3  | 801.3  | 126.1   | 153.5   | 155.5   | -- | *    |
| DYNLL1  | 924.6  | 604.5  | 800    | 121.2   | 139.5   | 151.1   | -- | *    |
| YBX1    | 1462.1 | 909.7  | 1116.7 | 175.5   | 221.6   | 222.5   | -- | *    |
| AK6     | 621.1  | 572    | 663.2  | 107.8   | 137.2   | 133.9   | -- | *    |
| K7FZ96  | 1308.6 | 1312.2 | 1399.2 | 285.9   | 298.2   | 290.4   | -- | *    |
| K7G186  | 123.7  | 117.1  | 121    | 22      | 29.1    | 28.6    | -- | *    |
| SARNP   | 648.1  | 490.9  | 706.5  | 121.2   | 146     | 154.2   | -- | *    |
| SYF2    | 553.3  | 420.7  | 443.2  | 91.2    | 98.8    | 136.1   | -- | *    |
| TCF4    | 188.4  | 173.9  | 146.9  | 38.1    | 40.6    | 40      | -- | *    |
| K7EXC8  | 45.4   | 28.5   | 33.7   | 3       | 13.2    | 9       | -- | *    |
| K7GE94  | 380.3  | 430.3  | 391.7  | 81      | 101.6   | 115.9   | -- | *    |
| DCK     | 821.1  | 745.4  | 836.6  | 182.3   | 212     | 203     | -- | *    |
| K7FRC0  | 880.7  | 720.9  | 911.2  | 209.1   | 216.6   | 214.8   | -- | *    |
| ACBD7   | 4991.7 | 4541.7 | 6223   | 1333.6  | 1398    | 1433    | -- | *    |
| GJC2    | 18.1   | 15.8   | 14.4   | 4.4     | 5.2     | 3.2     | -- | *    |

**Supplement Table S3**

| Protein | F1       | F2       | F3       | PM1     | PM2     | PM3     | Up | Down |
|---------|----------|----------|----------|---------|---------|---------|----|------|
| DYNLL1  | 90.5     | 59.1     | 78.1     | 590     | 1002.3  | 892.9   | *  | --   |
| K7FX63  | 86.2     | 59.6     | 68.2     | 665.3   | 728.1   | 793     | *  | --   |
| K7EXP1  | 42.3     | 46.8     | 55.7     | 365     | 510.2   | 554     | *  | --   |
| K7FHJ3  | 98.2     | 59.6     | 82.7     | 665.6   | 763.8   | 845.5   | *  | --   |
| K7G5Y2  | 38.4     | 36.5     | 50.7     | 322.7   | 353.7   | 461.8   | *  | --   |
| PFKP    | 44       | 24.7     | 38.2     | 268.6   | 245.5   | 301.2   | *  | --   |
| RBM45   | 89.2     | 83.2     | 77.7     | 570.7   | 607.9   | 589.3   | *  | --   |
| LDHA    | 316.7    | 243      | 262.7    | 1751.4  | 1980.5  | 1787.9  | *  | --   |
| K7EZ03  | 409.8    | 328.8    | 340.8    | 2333.3  | 2484.1  | 2409.9  | *  | --   |
| RNLS    | 59.8     | 59.1     | 76.1     | 418.7   | 438.3   | 426.6   | *  | --   |
| ATP6    | 29       | 21.6     | 40.3     | 197.7   | 186.4   | 201     | *  | --   |
| RBX1    | 223.7    | 185.5    | 253.5    | 1234.9  | 1466.9  | 1413.5  | *  | --   |
| PIH1D1  | 26       | 19.5     | 36.2     | 158.8   | 172.9   | 172.9   | *  | --   |
| K7G186  | 18.8     | 14.9     | 27.8     | 115.6   | 123.4   | 133     | *  | --   |
| CAMLG   | 42.7     | 25.2     | 48.2     | 214.6   | 234.2   | 248.7   | *  | --   |
| DENND4A | 11927.4  | 14986.1  | 12737.7  | 119.1   | 116.5   | 99.1    | -- | *    |
| EIF6    | 5740     | 5401.6   | 6056.9   | 204.3   | 177.8   | 200.8   | -- | *    |
| BRPF3   | 1190.5   | 1165.8   | 1287.6   | 44.4    | 41.3    | 44.4    | -- | *    |
| ARHGAP5 | 766.2    | 876.5    | 701.2    | 33.5    | 26.3    | 31.5    | -- | *    |
| POLR2H  | 2315.7   | 2168.3   | 1702.4   | 99.6    | 90.1    | 93      | -- | *    |
| HDGFL2  | 7703.2   | 7421.9   | 6167.5   | 369.1   | 339.4   | 333.7   | -- | *    |
| K7FDG6  | 566.9    | 688      | 778.5    | 37.9    | 32.5    | 32.8    | -- | *    |
| K7GG70  | 967516.7 | 1019208  | 1004697  | 58918.2 | 54339.6 | 55180.5 | -- | *    |
| K7GGJ3  | 134159.5 | 145429.5 | 169903.3 | 10049.1 | 9405    | 9237    | -- | *    |
| K7GID9  | 10766.8  | 13260.2  | 8832.9   | 729     | 724.7   | 748.2   | -- | *    |
| K7F1A5  | 922.4    | 800.5    | 416      | 56.4    | 46.6    | 46.5    | -- | *    |
| K7GEE1  | 885.3    | 949      | 615.5    | 57.8    | 60.6    | 56.8    | -- | *    |
| CEP20   | 1721.1   | 1878     | 1652.5   | 126.3   | 129.7   | 123.3   | -- | *    |
| ARL5A   | 814.9    | 974.2    | 606      | 66.3    | 56.7    | 59.7    | -- | *    |
| PM20D2  | 17025.8  | 16955    | 14568.5  | 1226.7  | 1288.2  | 1331    | -- | *    |