

## **Supplementary Material**

# **Characterization and Beige Adipogenic Potential of Human Embryo White Adipose Tissue-Derived Stem Cells**

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## Supplementary materials:

Currently, there is no method for the internal standardization of genes to be standardized between different specimens. Therefore, the researcher will select the most appropriate internal reference gene according to the type of cells and tissues and the experimental requirements. The internal reference gene must ensure that the stable expression level is similar to the target gene and has good efficiency. In order to guarantee the internal reference we used is a stable and effective internal reference for the sample of human WAsc, we screened and verified 5 pairs of internal reference(all of these internal references have been used in research articles):

1.  $\beta$ -actin (PrimerBank ID: 4501885a1)

Forward: CATGTACGTTGCTATCCAGGC

Reverse: CTCCTTAATGTCACGCACGAT

2. GAPDH (doi:10.1038/nm.4031)

Forward: CCACATCGCTCAGACACCAT

Reverse: GGCAACAATATCCACTTTACCAGAGT

3. GAPDH (PrimerBank ID: 126273608c1)

Forward: TGTGGGCATCAATGGATTTGG

Reverse: ACACCATGTATTCCGGGTCAAT

4. RPLP0 (PrimerBank ID: 49087144c1)

Forward: AGCCCAGAACACTGGTCTC

Reverse: ACTCAGGATTTCAATGGTGCC

5. Cyclophilin A (dio: 10.1038/srep39193)

Forward: TGTGTCAGGGTGGTGACTTC

Reverse: GTCTTGGCAGTGCAGATGAA

Then, we set 5 concentration gradients of cDNA from human WAsc to verify the stability of the internal reference gene.

The results are as follows:

| Internal reference                                 |                      | CT       |          |          | average |
|----------------------------------------------------|----------------------|----------|----------|----------|---------|
| NO.                                                | Concentration(ng/ul) | repeat-1 | repeat-2 | repeat-3 |         |
| 1. $\beta$ -actin<br>(PrimerBank ID:<br>4501885a1) | 2000                 | 14.99    | 15.7     | 15.21    | 15.30   |
|                                                    | 1000                 | 15.12    | 14.53    | 16.34    | 15.33   |
|                                                    | 500                  | 16.03    | 16.39    | 17.22    | 16.55   |
|                                                    | 250                  | 17.65    | 17.17    | 16.98    | 17.27   |
|                                                    | 125                  | 18.67    | 18.25    | 17.86    | 18.26   |
|                                                    |                      | CT       |          |          | average |
|                                                    | Concentration(ng/ul) | repeat-1 | repeat-2 | repeat-3 |         |
| 2.GAPDH<br>(doi:10.1038/nm.4031)                   | 2000                 | 14.21    | 14.5     | 14.22    | 14.31   |
|                                                    | 1000                 | 15.69    | 15.51    | 15.33    | 15.51   |
|                                                    | 500                  | 16.19    | 16.77    | 16.55    | 16.50   |
|                                                    | 250                  | 17.33    | 17.33    | 17.35    | 17.34   |
|                                                    | 125                  | 18.05    | 18.05    | 18.16    | 18.09   |
|                                                    |                      | CT       |          |          | average |
|                                                    | Concentration(ng/ul) | repeat-1 | repeat-2 | repeat-3 |         |

|                                             |                      |          |          |          |         |
|---------------------------------------------|----------------------|----------|----------|----------|---------|
| 3.GAPDH<br>(PrimerBank ID:<br>126273608c1)  | 2000                 | 13.85    | 14.15    | 14.32    | 14.11   |
|                                             | 1000                 | 15.53    | 14.84    | 15.01    | 15.13   |
|                                             | 500                  | 16.29    | 16.04    | 16.32    | 16.22   |
|                                             | 250                  | 17.26    | 17.36    | 17.25    | 17.29   |
|                                             | 125                  | 18.06    | 18.04    | 18.12    | 18.07   |
|                                             |                      | CT       |          |          | average |
|                                             | Concentration(ng/ul) | repeat-1 | repeat-2 | repeat-3 |         |
| 4.RPLP0<br>(PrimerBank ID:<br>49087144c1)   | 2000                 | 14.53    | 14.39    | 14.56    | 14.49   |
|                                             | 1000                 | 15.54    | 15.62    | 15.66    | 15.61   |
|                                             | 500                  | 16.91    | 16.78    | 16.88    | 16.86   |
|                                             | 250                  | 17.26    | 16.57    | 17.21    | 17.01   |
|                                             | 125                  | 18.37    | 18.01    | 18.65    | 18.37   |
|                                             |                      | CT       |          |          | average |
|                                             | Concentration(ng/ul) | repeat-1 | repeat-2 | repeat-3 |         |
| 5.Cyclophilin A<br>(dio: 10.1038/srep39193) | 2000                 | 14.86    | 14.94    | 14.82    | 14.87   |
|                                             | 1000                 | 15.99    | 15.62    | 15.87    | 15.83   |
|                                             | 500                  | 16.89    | 16.83    | 16.91    | 16.88   |
|                                             | 250                  | 17.41    | 17.33    | 17.35    | 17.36   |
|                                             | 125                  | 18.23    | 18.33    | 18.31    | 18.66   |

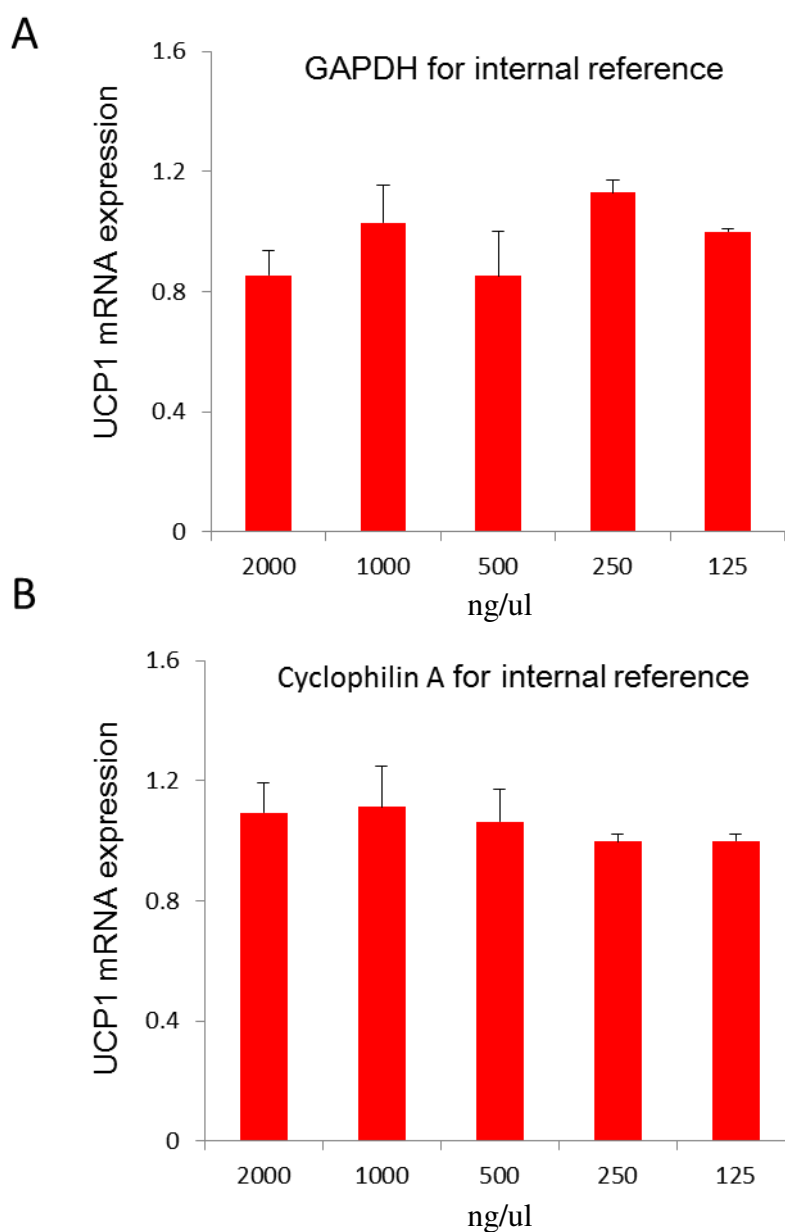
From the above results, it can be found that the expression levels of the internal reference No. 1, 3, and 4 are not sufficiently stable (marked in yellow) in human WAsc, and the difference in CT values in the same concentration group exceeds 0.5.

Then, we continued the validation with internal reference No. 2 and No. 5 by observing the expression of UCP1 in 5 concentration gradients of cDNA from human WAsc.

The results are as follows:

|                 |                      | CT                |          |          | average   |       |            |          |
|-----------------|----------------------|-------------------|----------|----------|-----------|-------|------------|----------|
|                 | Concentration(ng/ul) | repeat-1          | repeat-2 | repeat-3 |           |       |            |          |
| 2.GAPDH         | 2000                 | 14.21             | 14.5     | 14.22    | 14.31     |       |            |          |
|                 | 1000                 | 15.69             | 15.51    | 15.33    | 15.51     |       |            |          |
|                 | 500                  | 16.19             | 16.77    | 16.55    | 16.50     |       |            |          |
|                 | 250                  | 17.33             | 17.33    | 17.35    | 17.34     |       |            |          |
|                 | 125                  | 18.05             | 18.05    | 18.16    | 18.09     |       |            |          |
| UCP1            | 2000                 | 23.33             | 23.55    | 23.75    | 23.54     |       |            |          |
|                 | 1000                 | 24.33             | 24.56    | 24.55    | 24.48     |       |            |          |
|                 | 500                  | 25.77             | 25.65    | 25.35    | 25.59     |       |            |          |
|                 | 250                  | 26.25             | 26.13    | 26.08    | 26.15     |       |            |          |
|                 | 125                  | 27.05             | 27.06    | 27.12    | 27.08     |       |            |          |
|                 |                      | $\Delta\Delta$ CT |          |          |           |       |            |          |
| UCP1/GAPDH      | Concentration(ng/ul) | repeat-1          | repeat-2 | repeat-3 | AVERAGE   | group | Normalized | SE       |
|                 | 2000                 | 0.001797          | 0.001887 | 0.001353 | 0.0016788 | 2000  | 0.85352619 | 0.083947 |
|                 | 1000                 | 0.002507          | 0.001887 | 0.001677 | 0.0020234 | 1000  | 1.02870421 | 0.126653 |
|                 | 500                  | 0.001307          | 0.002123 | 0.002244 | 0.0018909 | 500   | 0.85134878 | 0.149593 |
|                 | 250                  | 0.002064          | 0.002244 | 0.002355 | 0.002221  | 250   | 1.12919273 | 0.043032 |
|                 | 125                  | 0.001953          | 0.00194  | 0.002008 | 0.0019669 | 125   | 1          | 0.010635 |
|                 |                      |                   |          |          |           |       |            |          |
|                 |                      | CT                |          |          | average   |       |            |          |
|                 | Concentration(ng/ul) | repeat-1          | repeat-2 | repeat-3 |           |       |            |          |
| 5.Cyclophilin A | 2000                 | 14.86             | 14.94    | 14.82    | 14.87     |       |            |          |
|                 | 1000                 | 15.99             | 15.62    | 15.87    | 15.83     |       |            |          |

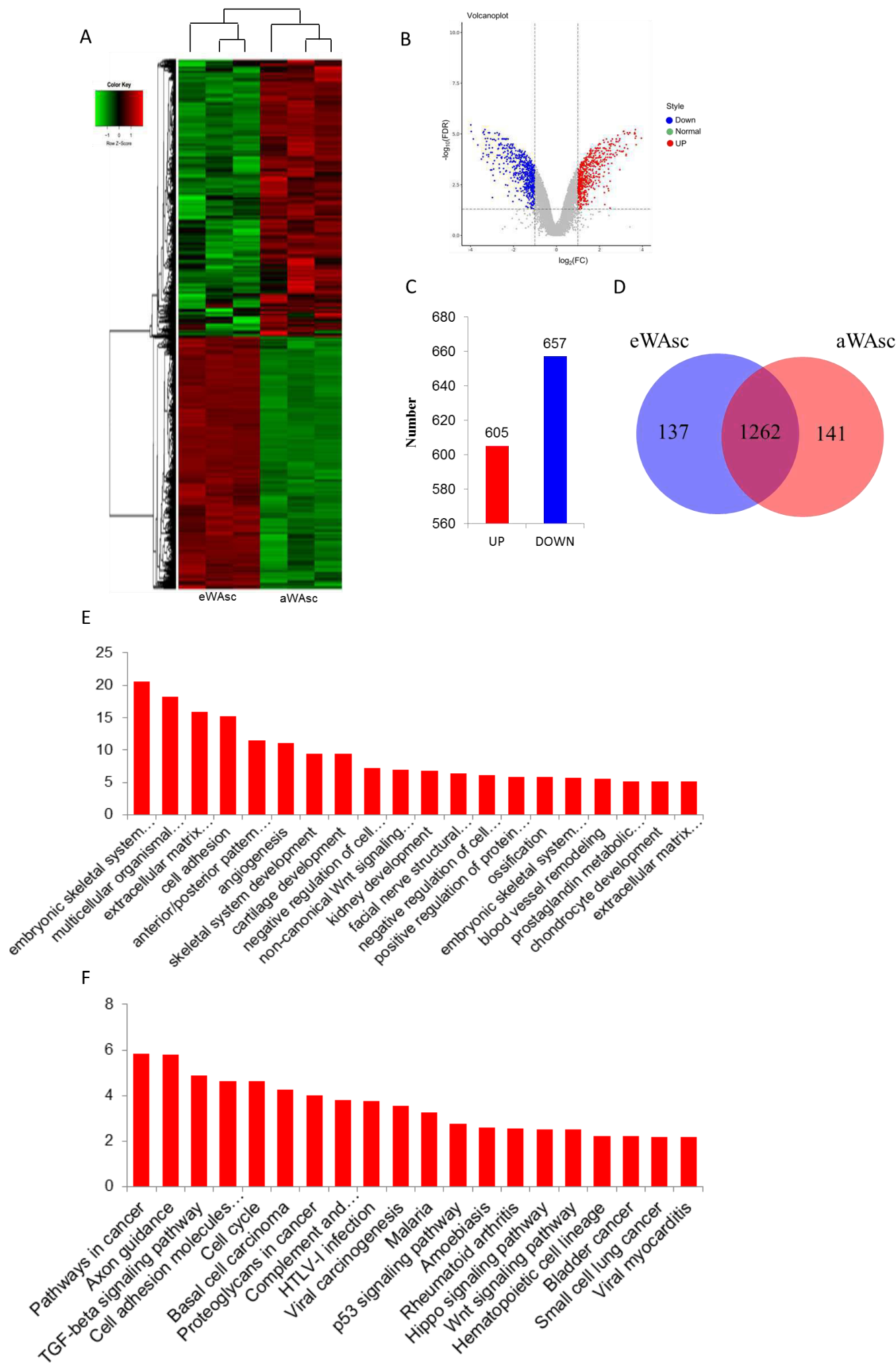
|            |                      |                   |          |          |           |       |            |          |
|------------|----------------------|-------------------|----------|----------|-----------|-------|------------|----------|
|            | 500                  | 16.89             | 16.83    | 16.91    | 16.88     |       |            |          |
|            | 250                  | 17.41             | 17.33    | 17.35    | 17.36     |       |            |          |
|            | 125                  | 18.23             | 18.33    | 18.31    | 18.29     |       |            |          |
| UCP1       | 2000                 | 23.33             | 23.55    | 23.75    | 23.54     |       |            |          |
|            | 1000                 | 24.33             | 24.56    | 24.55    | 24.48     |       |            |          |
|            | 500                  | 25.77             | 25.65    | 25.35    | 25.59     |       |            |          |
|            | 250                  | 26.25             | 26.13    | 26.08    | 26.15     |       |            |          |
|            | 125                  | 27.05             | 27.06    | 27.12    | 27.08     |       |            |          |
|            |                      | $\Delta\Delta$ CT |          |          |           |       |            |          |
| UCP1/GAPDH | Concentration(ng/ul) | repeat-1          | repeat-2 | repeat-3 | AVERAGE   | group | Normalized | SE       |
|            | 2000                 | 0.00282           | 0.002559 | 0.00205  | 0.0024766 | 2000  | 1.09328582 | 0.099805 |
|            | 1000                 | 0.003086          | 0.002036 | 0.002438 | 0.0025201 | 1000  | 1.11249562 | 0.135028 |
|            | 500                  | 0.002123          | 0.002213 | 0.002879 | 0.0024049 | 500   | 1.06397116 | 0.105374 |
|            | 250                  | 0.002182          | 0.002244 | 0.002355 | 0.0022603 | 250   | 0.9977979  | 0.02234  |
|            | 125                  | 0.002213          | 0.002355 | 0.002228 | 0.0022653 | 125   | 1          | 0.019923 |



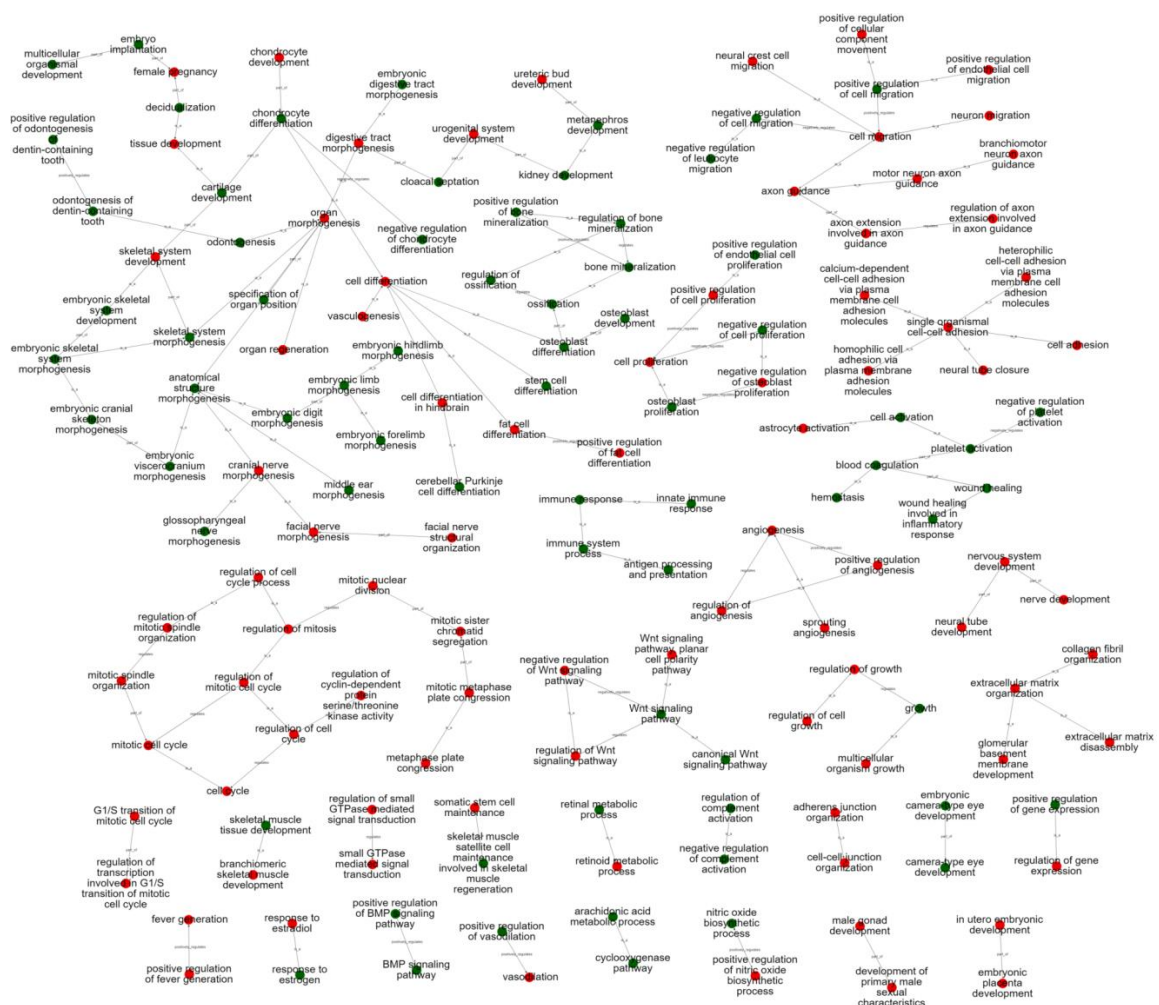
We know that the same sample is assigned to different concentrations, but the expression of UCP1 should be still consistent. From the above results, we can see that the expression levels of UCP1 with the internal reference No. 5 (Cyclophilin A) are more sufficiently stable than with

the internal reference NO.2 under different concentration gradients of cDNA from human WAsc (figure A and B). Therefore, we use Cyclophilin A as an internal reference for this study.

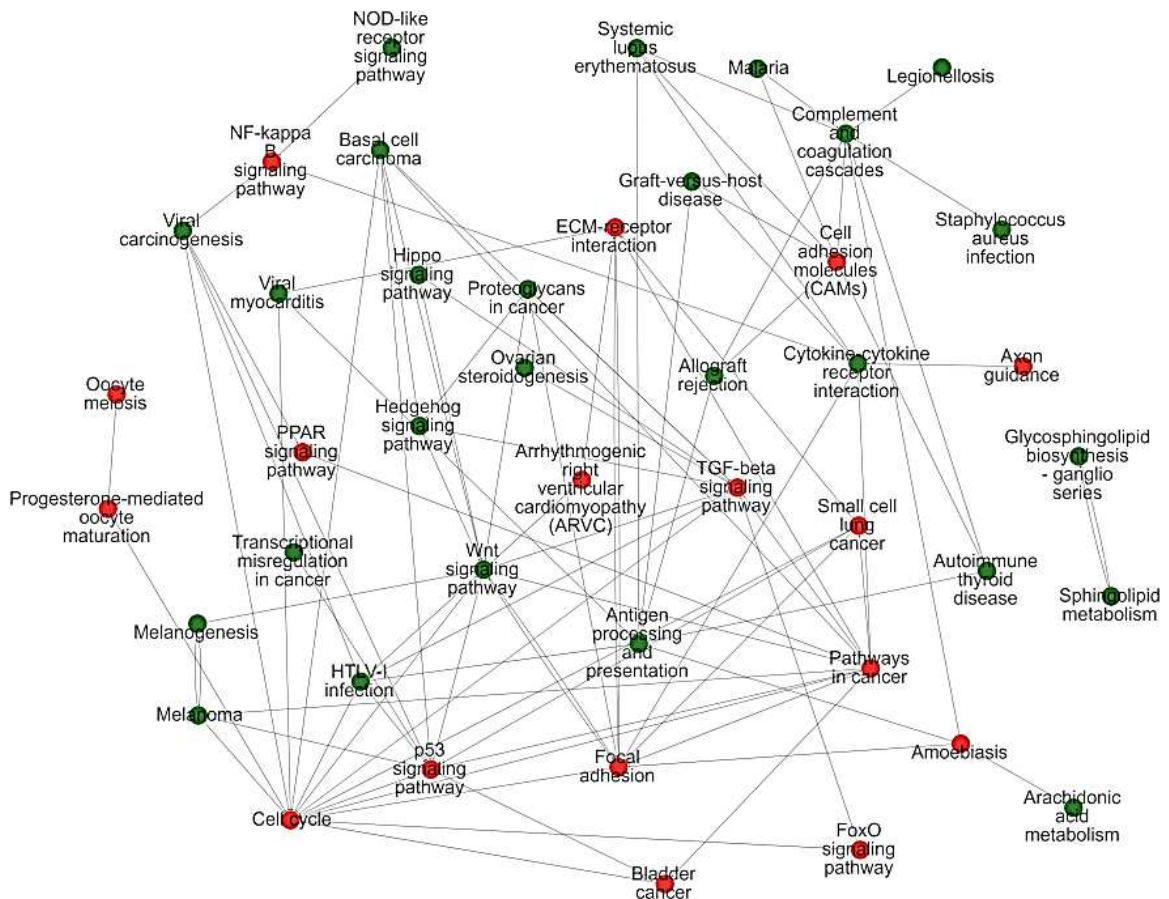
Supplementary Fig. S1



G



H



### **Figure S1. DEGs, GO, and KEGG analysis.**

Based on the normalized FPKM, heatmap (A), and volcano-plot (B), there were 1263 DEGs that were differentially expressed between the aWAsc and eWAsc groups, composed of 605 upregulated and 657 downregulated genes in eWAsc compared to aWAsc (C). Venn diagrams of eWAsc versus aWAsc. We identified 137 specific genes only expressed in eWAsc and 141 specific genes only expressed in aWAsc (D). Significant GO analysis (E) and KEGG analysis (F) results were calculated using the Fisher Exact Test. Red indicates significantly enriched pathways and GO terms. Node size represents the node degree. (G) GO-Tree: red dots represent the upregulated GO terms and green dots represent the downregulated GO terms. (H) Pathway-act-network. Red dots represent the upregulated pathway terms and green dots represent the downregulated pathway terms.