Supplementary Tables and Figures

Table S1. Primers used for RT-qPCR analysis[1]

Target gene	Forward primer (5'-3')	Reverse primer (5'-3')	References
CDK1	TTTTCAGAGCTTTGGGCACTC	TTTTCGAGAGCSGATCCAAGC	[1]
p21	AGGACCATGTGGACCTGTTG	TTAGGGCTTCCTCTTGGAGA	[2]
CDK4	GCATCCCAATGTTGTCCG	GGGGTGCCTTGTCCAGATA	[3]
Caspase 2	GCGACGCTGGCAAAGAG	CCTGAGGGCGAGGAACA	XM_005657720.3
Caspase 3	GCCATGGTGAAGAAGGAAAA	GTCCGTCTCAATCCCACAGT	[3]
Caspase 8	TCCTGAGCCTGGACTACAT	CTCCTCCTCATTGGTTTCC	[3]
OGA	AAAAGCATGATGGCTTGCCT	CCACCCACCAGGTGAAGTTAA	[5]
OGT	AGCGGGACTCAATTACCCTTT	ATTGTCTGGCTCTTGTCTCCA	[5]
IL-6	AATGTCGAGGCTGTGCAGATT	TGGTGGCTTTGTCTGGATTCT	NM_214399.1
CLDN1	AGCTGTGCATGGCCTCTTGT	CCAATGTCAATGGCAACACCCT	[7]
ZO-1	CGGAACTATGACCATCGCCTAC	CTTCGGGATGTTGTCTGGAGTC	[7]
TGR5	CCATGCACCCCTGTTGCT	GGTGCTGTTGGGTGTCATCTT	XM_013984487.2
LXRα	AGAACAGATCCGCCTGAAGA	GGTCTGAAAAGGAGCGTCTG	XM_013994348.2
FXR	TATGAACTCAGGCGAATGCCTGCT	ATCCAGATGCTCTGTCTCCGCAAA	[8]
Bax	CCGAAATGTTTGCTGACG	AGCCGATCTCGAAGGAAGT	[4]
Bcl-2	ACCTGAATGACCACCTAGAGC	TCCGACTGAAGAGCGAAC	[4]
ND1	TCAACCCTAGCAGAAACCAACGA	AAGAATATGGCGAAAGGTCGGCT	[10]
ND2	TTTCCTAACACAAGCCACAGCCTC	ATGCCTTGGGTTACTTCTGGGACT	[10]
ND3	AGCACGCCTCCCATTCTCAAT	TGCTAGGCTTGCTGCTAGTAGG	[11]
ND4	TCGCCTATTCATCAGTAAGTCA	GGATTATGGTTCGGCTGTGTA	[11]
ATP6	TACCACACTCATTCACACCCACCA	TGTTCCTTGTGGTAGAAAGTGGGC	[10]
ATP8	TGCCACAACTAGATACATCC	GCTTGCTGGGTATGAGTAG	[11]
COX2	TGGCTTACCCTTTCCAACTAGGCT	TTGGGCATCCATTGTGCTAGTGTG	[10]
COX3	ACACCCGAATTAGGAGGTTGCTGA	TACGCCTAGTGCAATGGTGATGGA	[10]
Cytb	TCACACGATTCTTCGCCTTCCACT	TAGGGTTGTTGGATCCGGTTTCGT	[10]
D-loop	ACACACCCTATAACGCCTTGCC	GGGTAGGTGCCTGCTTTCGTAG	[11]
β-actin	ATGCTTCTAGACGGACTGCG	GTTTCAGGAGGCTGGCATGA	[9]
GAPDH	GCTTGTCATCAATGGAAAGG	CATACGTAGCACCAGCATCA	[6]

[1] IL-6, interleukin-6; CDK, cyclin-dependent kinase; ZO-1, zonula occludens-1; CLDN1, claudin 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OGA, O-GlcNAcase; OGT, O-GlcNAc Transferase; Bax, BCL2-associated X; Bcl-2, B-cell lymphoma-2; FXR, farnesoid X receptor; LXRα, liver X receptor α; TGR5, G-coupled protein receptor; ND1, NADH dehydrogenase subunit 1; ND2, NADH dehydrogenase subunit 2; ND3, NADH dehydrogenase subunit 3; ND4, NADH dehydrogenase subunit 4; COX2, cytochrome c oxidase subunit 2; COX3, cytochrome c oxidase subunit 3; Cytb, cytochrome b; ATP6, ATP synthase F0 subunit 6; ATP8, ATP synthase F0 subunit 8.

Table S2. The raw data from RNA-Sequencing analysis of CON and CDCA groups

Group	Sample ID	Raw reads	Clean reads	Total mapped	Multiple mapped	Uniquely mapped	GC content (%)	Q20(%)	Q30 (%)
CON	CON_3	65,466,558	64,675,190	61,746,328(95.47%)	5,768,052(8.92%)	55,978,276(86.55%)	51.99	98.2	94.76
	CON_2	69,271,904	68,540,858	65,644,376(95.77%)	4,686,129(6.84%)	60,958,247(88.94%)	51.46	98.29	94.97
	CON_1	61,354,956	60,811,650	58,355,295(95.96%)	4,267,914(7.02%)	54,087,381(88.94%)	50.91	98.39	95.2
CDCA	CDCA_3	65,356,232	64,745,300	61,984,695(95.74%)	3,125,867(4.83%)	58,858,828(90.91%)	51.37	98.4	95.25
	CDCA_2	61,757,660	61,112,694	58,433,389(95.62%)	2,999,136(4.91%)	55,434,253(90.71%)	51.75	98.33	95.08
	CDCA_1	60,683,302	60,088,126	57,502,899(95.7%)	2,907,690(4.84%)	54,595,209(90.86%)	51.48	98.34	95.1

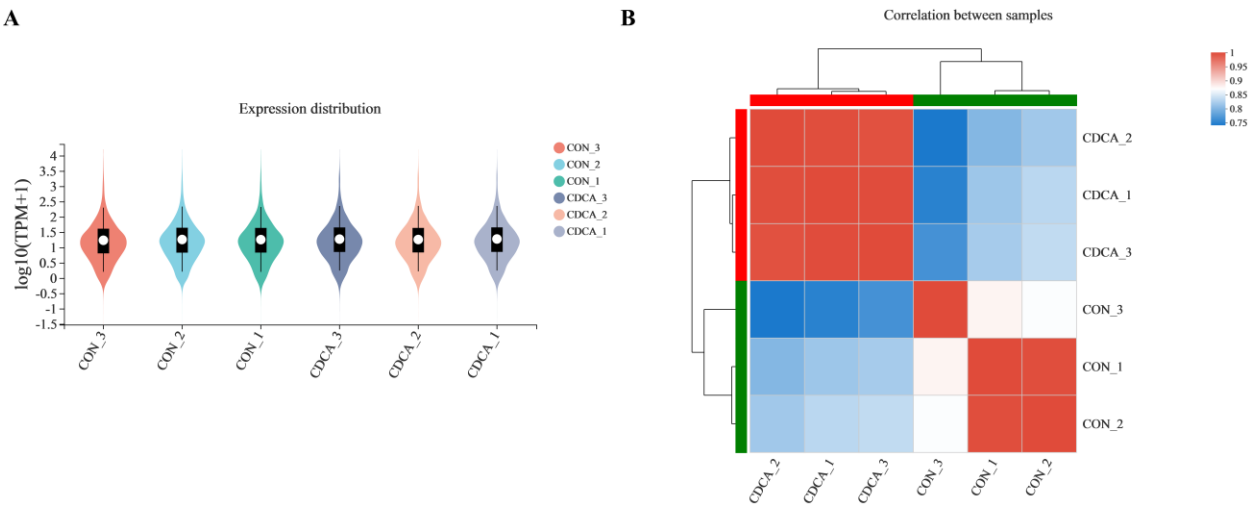

Figure S1. Outlier Box Plots to identify biological outliers (A). All the samples had a similar distribution of the data and none was considered an outlier; The results of correlation analysis between samples (B). Results showed that biological replicates from the two groups could be well distinguished and the experimental design was reasonable. These results confirmed the high reproducibility of the sequencing data.

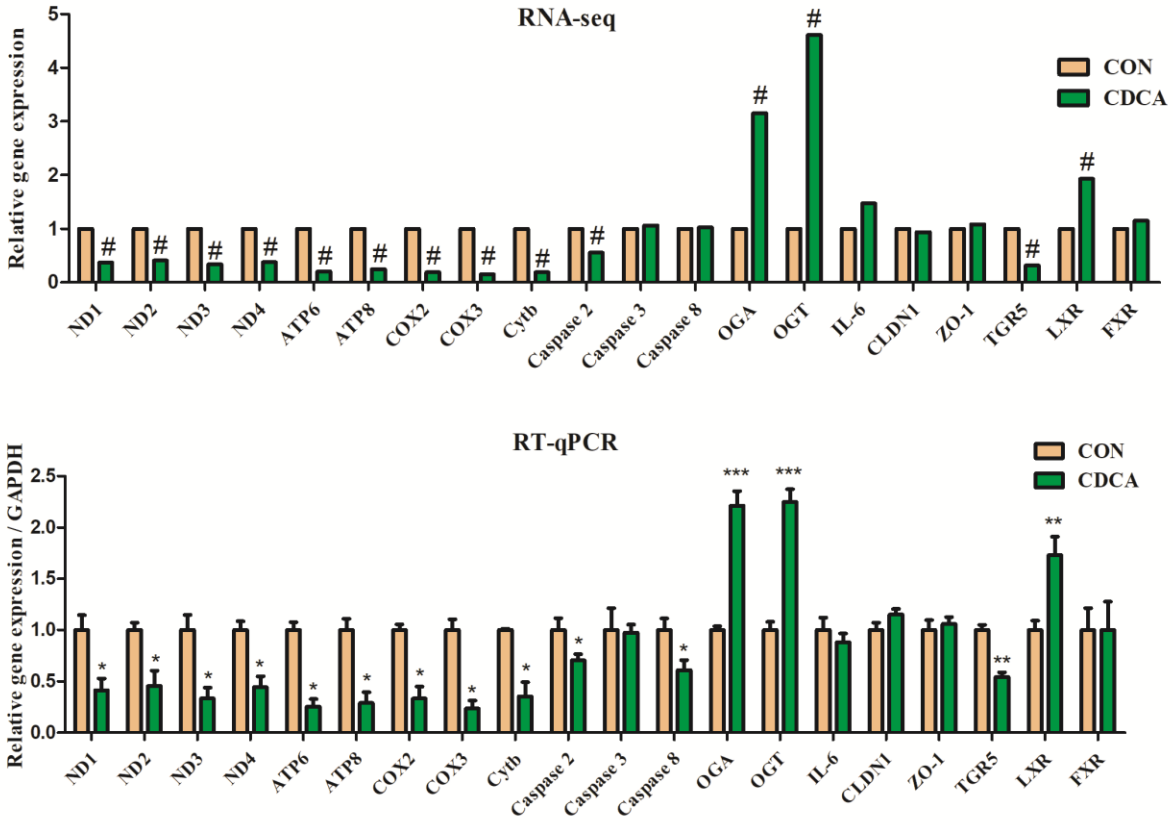

Figure S2. Validation of transcriptomic results by RT-qPCR in IPEC-J2 cell. The 3 upregulated DEGs, 11 downregulated DEGs, and 6 unchanged genes observed by RNA-seq were selected to validate using RT-qPCR. All validated genes have similar trends in expression when comparing RNA-seq with RT-qPCR. # FDR < 0.05, * p < 0.05, ** p < 0.01, *** p < 0.001, versus CON group. IL-6, interleukin-6; CDK, cyclin-dependent kinase; ZO-1, zonula occludens-1; CLDN1, claudin 1; OGA, O-GlcNAcase; OGT, O-GlcNAc Transferase; Bax, BCL2-associated X; Bcl-2, B-cell lymphoma-2; FXR, farnesoid X receptor; LXRα, liver X receptor α; TGR5, G-coupled protein receptor; ND, NADH dehydrogenase subunit; COX, cytochrome c oxidase subunit; Cytb, cytochrome b; ATP, ATP synthase F0 subunit.

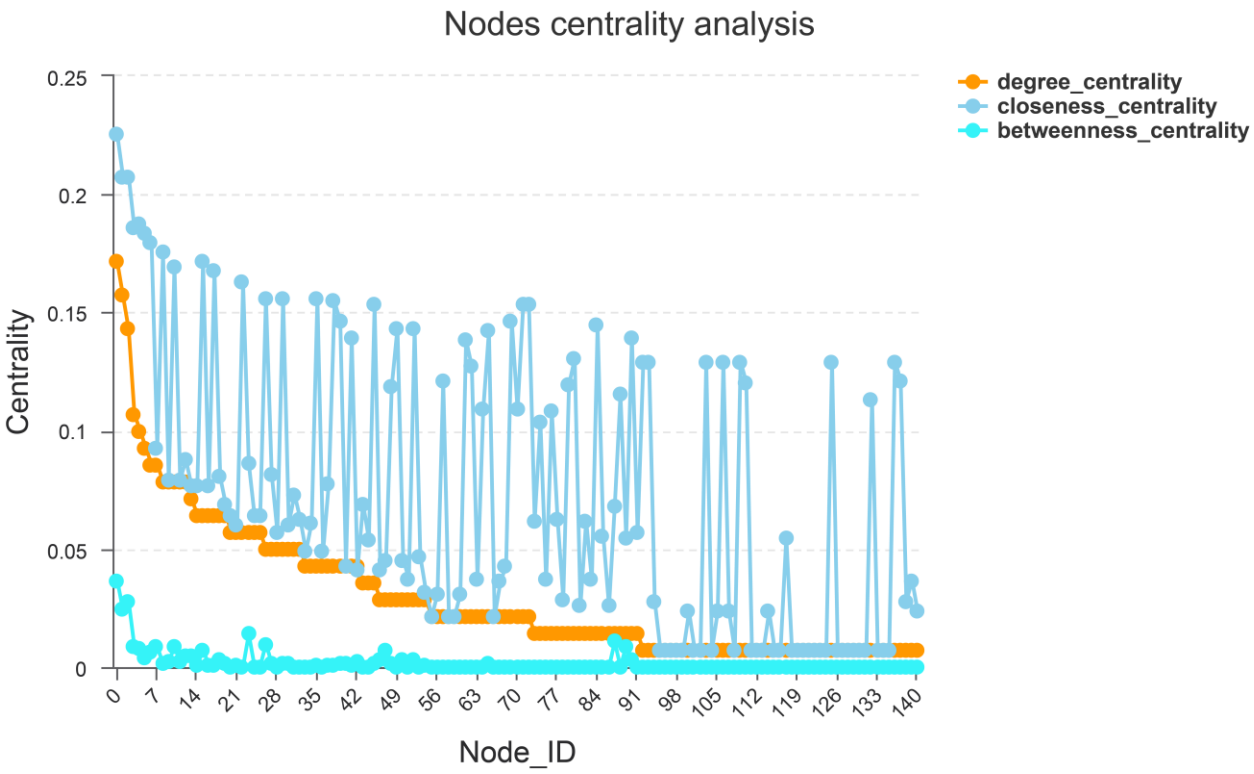

Figure S3. The nodes centrality analysis for protein-protein interaction (PPI) network. Three centrality methods including degree, closeness, and betweenness centrality were used to explore the key genes in the PPI network. The detailed information for the centrality degree is shown in Table S6.

References

1. Lee J., You J., Lee G.S., Hyun S.H. & Lee E. (2013) Pig Oocytes With a Large Perivitelline Space Matured In Vitro Show Greater Developmental Competence After Parthenogenesis and Somatic Cell Nuclear Transfer. Molecular Reproduction and Development 80, 753-62.
2. Yin B.X., Ren H.Y., Cai H., Jiang Y.Q., Zhao S.H. & Wang H. (2020) Dynamics of cardiomyocyte and muscle stem cell proliferation in pig. Experimental Cell Research 388.
3. Ding H.X., Yang Y.Z., Wei S.L., Spicer L.J., Kenez A., Xu W., Liu Y. & Feng T. (2021) Influence of N-acetylcysteine on steroidogenesis and gene expression in porcine placental trophoblast cells. Theriogenology 161, 49-56.
4. Zhu L.H., Cai X., Guo Q., Chen X.L., Zhu S.W. & Xu J.X. (2013) Effect of N-acetyl cysteine on enterocyte apoptosis and intracellular signalling pathways' response to oxidative stress in weaned piglets. British Journal of Nutrition 110, 1938-47.
5. Shibutani M., Mori T., Miyano T. & Miyake M. (2015) Removal of O-GlcNAcylation is important for pig preimplantation development. Journal of Reproduction and Development 61, 341-50.
6. Cai L., Wei Z.X., Zhao X.M., Li Y.P., Li X.L. & Jiang X.R. (2022) Gallic acid mitigates LPS-induced inflammatory response via suppressing NF-kappa B signalling pathway in IPEC-J2 cells. Journal of Animal Physiology and Animal Nutrition 106, 1000-8.
7. Huang S.M., Wu Z.H., Li T.T., Liu C., Han D.D., Tao S.Y., Pi Y., Li N. & Wang J.J. (2020) Perturbation of the lipid metabolism and intestinal inflammation in growing pigs with low birth weight is associated with the alterations of gut microbiota. Science of the Total Environment 719.
8. Radtke J., Geissler S., Schutkowski A., Brandsch C., Kluge H., Duranti M.M., Keller S., Jahreis G., Hirche F. & Stangl G.I. (2014) Lupin protein isolate versus casein modifies cholesterol excretion and mRNA expression of intestinal sterol transporters in a pig model. Nutrition & Metabolism 11.
9. Lin M., Zhang B.L., Yu C.N., Li J.L., Zhang L., Sun H., Gao F. & Zhou G.H. (2014) L-Glutamate supplementation improves small intestinal architecture and enhances the expressions of jejunal mucosa amino acid receptors and transporters in weaning piglets. Plos One 9.
10. Weller M.M.D.C.A., Alebrante L., Campos P.H.R.F., Saraiva A., Silva B.A.N., Donzele J.L., et al. Effect of heat stress and feeding phosphorus levels on pig electron transport chain gene expression. Animal 2013;7(12):1985-93.
11. Jia Y, Song H, Gao G, Cai D, Yang X, Zhao R. Maternal betaine supplementation during gestation enhances expression of mtDNA-encoded genes through D-loop DNA hypomethylation in the skeletal muscle of newborn piglets. J Agric Food Chem 2015;63(46):10152-60.