

Front. Agr. Sci. Eng.

<https://doi.org/10.15302/J-FASE-2025637>

Verso: Front. Agr. Sci. Eng.

Recto: Jiaping LI et al. Enhanced adipogenesis and fatty acid transport in Tibetan pigs compared to Duroc pigs

**RESEARCH ARTICLE**

# Enhanced adipogenesis and fatty acid transport in Tibetan pigs compared to Duroc pigs: insights from cellular heterogeneity by scRNA-seq

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Received January 21, 2025;

Accepted April 24, 2025.

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## **SUPPLEMENTARY MATERIALS:**

Tables S1–S2; Figs. S1–S4

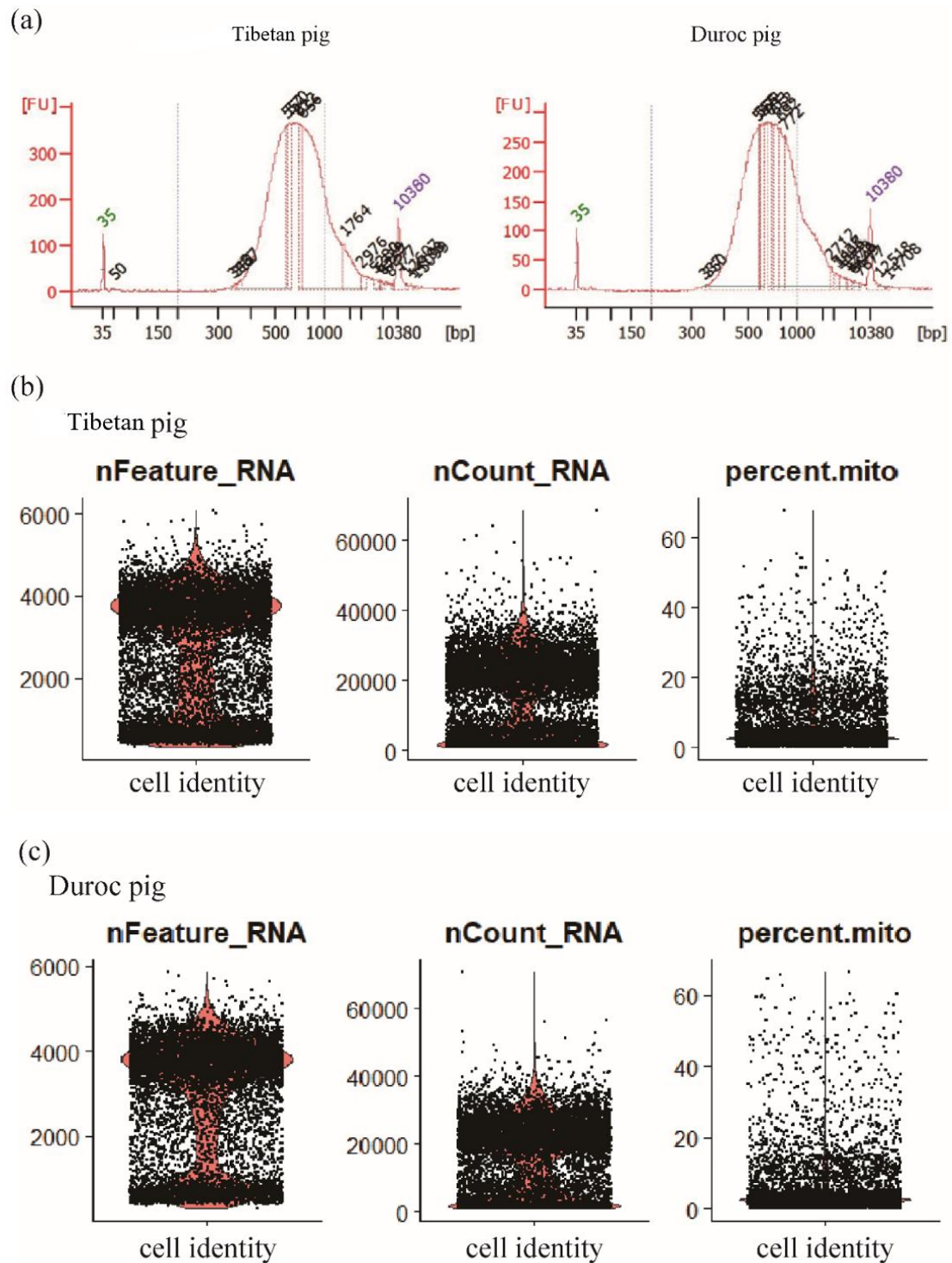
**Table S1** The primers of genes used for qPCR in the study

Gene	Forward ( 5'-3' )	Reverse ( 5'-3' )
<i>18S</i>	GGCCGTTCTTAGTTGGTGGA	CCCGGACATCTAAGGGCATC
<i>Adipoq</i>	CTTCCACGTACGGTCTACT	TCTCTTCATCCCCGTATGCC
<i>Cebpa</i>	CTCACCGCTCCGATTCTTAC	TCCTTCTATTGCGGGGGAGA
<i>Cebpβ</i>	CAACCTGGAGACGCAGCATA	GCTTGAACAAGTCCGCAGG
<i>Cenpf</i>	TTGCAACGAGACCCTCAGTC	AGCCGTTTCAGCTCGGATATG
<i>Cd36</i>	TGGGCTGCAATAGAGACTGT	TGCTGTAAACTTCCGTGCCT
<i>Cd142</i>	AAGCTGTGTCCCGAGAACTG	CGCTCCTTCTGCACCTTGTA
<i>Cox5b</i>	CTACTGATGAGGAGCAGGCG	TCCGCTTGTTGGTGATGGAG
<i>Cycs</i>	GTCTCTTTGGGCGGAAGACA	GTCTCCTCTCCCCAGGTGAT
<i>Dio2</i>	AGGAAGCAACAACCATGGAC	CAGGTAAGTCCCAACCATCT
<i>Fabp4</i>	TGAAAGAAGTGGGAGTGGGC	CTGGCCCAATTTGAAGGCAA
<i>Glut4</i>	TGGACGGTTCCTCATTGGTG	GTGATGCCAGGAGTAGTGG
<i>Leptin</i>	CCAGTTTACGGTTGAATGCC	CGGCATTTATCACCGGCTTT
<i>Mki67</i>	TGAGGCCCGAGTTATTCGATG	GATGGCTGAGGCTTGATGATT
<i>Ndufa6</i>	CTCTATCGCGCCTGGTATCG	TGTGACGTGGGCATTCTTCA
<i>Ndufs5</i>	TGGAATCGGTGGTATCCGTG	TTAGCTTATCCCCGTGCCCTC
<i>Ndufv3</i>	GGAGGAGTATTTTCGCGGACTC	TCCTGTGCCGCCACTACAT
<i>Pparg</i>	AACATTTACAAGAGGTGACCA	CTCTCGGGAATGGGATGTCT
<i>Tubb6</i>	ATCGGCACCAAGTTTGGGA	GGGCACGTATTTCTGAGACGA
<i>Ucp3</i>	TCACCTTCAGGACACGTTCG	AGGCATCCATCCTAGTGGGT

**Table S2** Cell viability of SVF cell suspension before single-cell RNA-seq experiment and quality control parameter of sequencing in Tibetan and Duroc pigs

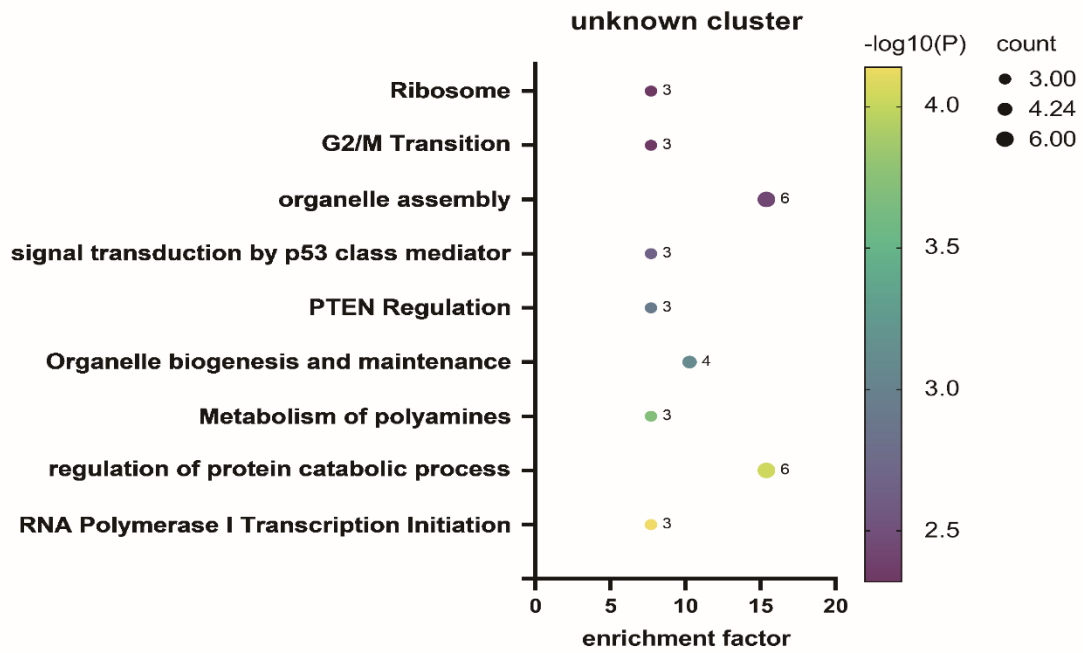
Quality control parameter	Tibetan pig	Duroc pig
cell viability	95%	86%
Estimated number of cells	9874	9051
Number of reads	493,883,161	482,743,127
Mean reads per cell	50,018	53,335
Median genes per cell	3236	3504
Total genes detected	16,352	16,090
Median UMI counts per cell	17,179	19,532

Note: The cell viability data was measured by Countess® II Automated Cell Counter and the quality control value of sequencing was from the BGI company.

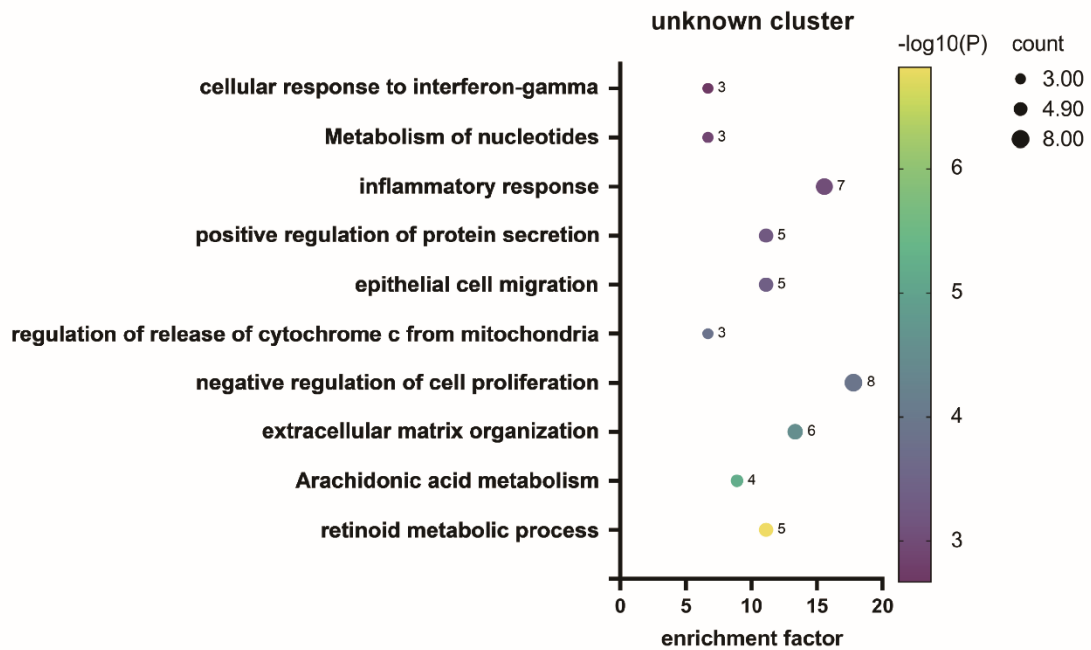


**Fig. S1** Quality control of cDNA library and scRNA-seq data of SVF cells in neck white adipose tissue from Tibetan and Duroc pigs. (a) Quality control of cDNA library by Agilent 2100 Bioanalyzer System detection from SVF cells in Tibetan and Duroc pigs. (b) Quality control of nFeature\_RNA, nCount\_RNA and percent. Mito (percentage of mitochondrial genes) in each cell from SVF cells of in Tibetan pigs by scRNA-seq analysis. (c) Quality control of nFeature\_RNA, nCount\_RNA and percent. Mito (percentage of mitochondrial genes) in each cell from SVF cells of in Duroc pigs by scRNA-seq analysis.

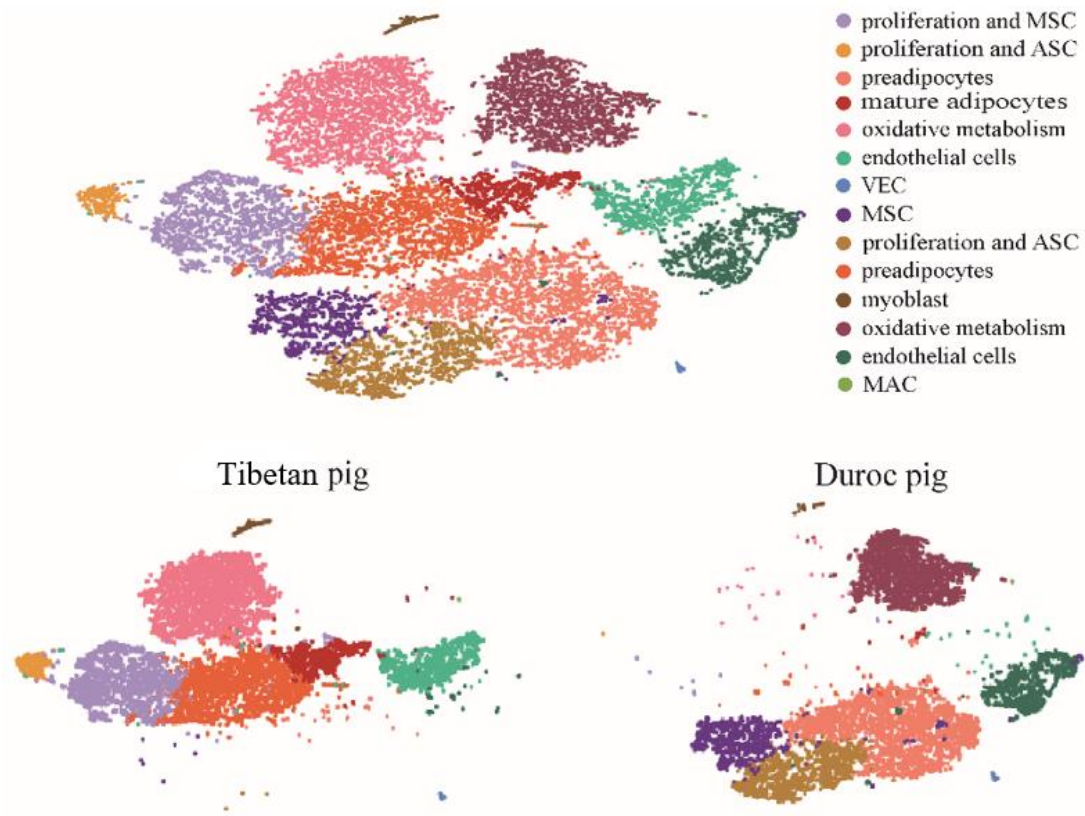
(a)



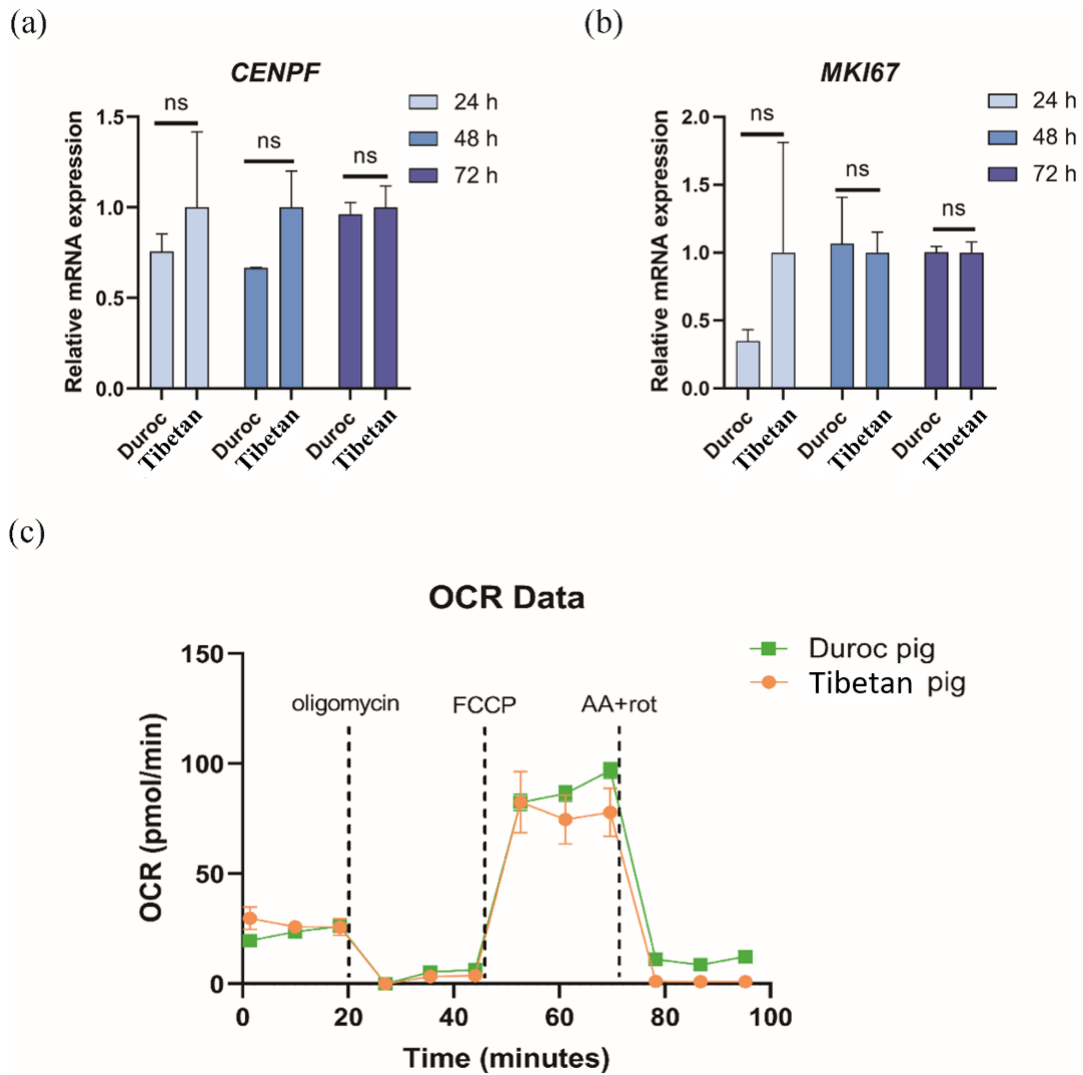
(b)



**Fig. S2** Augmented pathway of unknow clusters by GO analysis in scRNA-seq analysis from Tibetan and Duroc pigs. (a) GO analysis of differentially expressed genes in unknown cluster of scRNA-seq analysis in Tibetan pigs. (b) GO analysis of differentially expressed genes in unknown cluster of scRNA-seq analysis in Duroc pigs.



**Fig. S3** Aggregated t-SNE plot of 18,925 SVF cells from neck white adipose tissue in Tibetan and Duroc pigs. t-SNE clustering revealed 14 clusters based on differently expressed genes. MSC, mesenchymal stem cells; ASC, adipocyte stem cells; MAC, macrophage; VEC, vascular endothelial cell.



**Fig. S4** Detection of proliferation genes in SVF cells and oxygen consumption rate in mature adipocytes at day 8 during adipocyte differentiation. (a) Expression of *Cenpf* in SVF cells at 24, 48, 72 h by qPCR detection in Tibetan and Duroc pigs.  $n = 3$  in each group. Statistical significance was calculated on these mean values by  $t$ -test,  $^{ns} P > 0.05$ . (b) Expression of *Mki67* in SVF cells at 24, 48, 72 h by qPCR detection in Tibetan and Duroc pigs.  $n = 3$  in each group. Statistical significance was calculated on these mean values by  $t$ -test,  $^{ns} P > 0.05$ . (c) Oxygen consumption rate of mature adipocytes on day 8 during adipocyte differentiation from Tibetan and Duroc pigs.  $n = 5$  in each group.