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Developmental transition of brown adipose tissue from a thermogenic to a lipogenic phenotype in neonatal goats revealed by single-nucleus transcriptomics

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ABSTRACT

Brown adipose tissue (BAT) is essential for neonatal thermogenesis and early-life metabolic adaptation. In goat neonates, BAT is primarily concentrated in the perirenal region and rapidly regresses during the first month after birth, yet the cellular and molecular programs driving this transition remain poorly defined. In the present study, a comprehensive single-nucleus transcriptomic atlas of perirenal adipose tissue was generated from 54 638 high-quality nuclei collected at postnatal days 0, 7, and 14. This resource resolved six transcriptionally distinct perirenal adipose cell populations and revealed pronounced developmental remodeling after birth. Adipocyte progenitors exhibited strong secretory signatures, indicating potential involvement of secretory programs in early postnatal adipose remodeling. Across early postnatal development, perirenal adipose tissue showed coordinated transcriptional remodeling in lipid and fatty acid metabolism, accompanied by marked changes in AMPK and PPAR signaling, indicating a progressive shift from a thermogenesis-associated program toward lipid storage. This transition was accompanied by expansion of adipocyte subpopulations associated with fat deposition and reduction of populations linked to thermogenic function. By postnatal day 14, perirenal adipose tissue had undergone a marked transition from a brown adipose phenotype toward a white-like adipose state. These findings provide insights into the early postnatal cellular trajectory of goat BAT and its role in neonatal thermogenesis and adipose tissue remodeling.

Keywords: Goat; Adipose tissue; High-thermogenic; High-fat deposition; Single-cell transcriptomics

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INTRODUCTION

Adipose tissue is a vital metabolic organ that coordinates energy storage, substrate utilization, endocrine signaling, and whole-body energy homeostasis (Sakers et al., 2022; Zhao et al., 2022a). Brown adipose tissue (BAT) is specialized for non-shivering thermogenesis (NST), a mitochondrial heat-generating process that protects mammals from hypothermia and contributes to metabolic regulation (Jun et al., 2023). During cold exposure, BAT rapidly increases heat production through enhanced mitochondrial uncoupling (Li et al., 2019). The identification of metabolically active brown adipocytes in adult humans has reinvigorated interest in BAT as a regulator of energy expenditure and metabolic health (Becher et al., 2021; Betz & Enerbäck, 2015; Gavaldà-Navarro et al., 2022; Li et al., 2019). In many mammals, including humans, goats, sheep, and rabbits, BAT is most abundant during early life, facilitating the transition of newborns from the thermally regulated uterine environment to the colder and more variable external environment (Li et al., 2019). Precise temporal control of NST is therefore essential, with rapid activation after birth required for neonatal thermal adaptation and suppression before birth necessary to prevent fetal overheating (Li et al., 2019). Vaginal delivery appears to provide critical perinatal cues for timely BAT activation in newborns. In sheep (*Ovis aries*), expression of the key thermogenic gene *UCP1* peaks on the first day after birth, whereas cesarean-born offspring show impaired thermoregulation, underscoring the importance of birth-associated BAT activation for neonatal thermal adaptation (Clarke et al., 1997; Li et al., 2019; Lomax et al., 2007). Functional BAT therefore enables young animals to withstand cold exposure and maintain core body temperature during early postnatal life (Symonds et al., 2012, 2018; Zhao et al., 2024).

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BAT is not developmentally static. During postnatal growth, BAT can undergo whitening, a remodeling process in which thermogenic adipocytes acquire a white adipose tissue (WAT)-like phenotype characterized by enlarged lipid droplets, reduced thermogenic capacity, and decreased expression of thermogenic genes such as *UCP1* (Kotzbeck et al., 2018; Shimizu et al., 2014). Histological analyses of human autopsy samples first demonstrated age-dependent morphological remodeling of BAT, with progressive lipid accumulation and gradual acquisition of WAT-like features (Heaton, 1972; Tanuma et al., 1975). Positron emission tomography (PET) studies subsequently confirmed that BAT activity declines with age, supporting a functional transition that parallels these structural changes (Gerngroß et al., 2017). Rapid postnatal BAT whitening has also been observed under normal physiological conditions in goats (Zhao et al., 2022b, 2024), sheep (Basse et al., 2015), and rabbits (Huang et al., 2022), indicating that early-life BAT remodeling occurs across several mammalian species.

Accumulating evidence indicates that BAT whitening is regulated by a multilayered molecular network involving transcription factors (Du et al., 2022b), microRNAs (miRNAs) (Liu et al., 2022), long non-coding RNAs (lncRNAs) (Liu et al., 2023), circular RNAs (circRNAs) (Du et al., 2022a), and small-molecule metabolites (Wang et al., 2021). Advances in next-generation sequencing have refined adipose tissue research beyond conventional methods, enabling higher-resolution analysis of cell-state diversity. Single-nucleus RNA sequencing (snRNA-seq) has resolved brown adipocyte subpopulations with high and low thermogenic activity and demonstrated temperature-responsive interconversion between these states (Song et al., 2020). *UCP1*-positive dormant brown adipocytes have also been identified in human perirenal adipose tissue (PRAT), although whether these cells correspond to low-thermogenic brown adipocytes in mouse BAT remains unclear (Jespersen et al., 2019). In rabbits, single-cell RNA-seq (scRNA-seq) revealed a progressive loss of brown adipocyte progenitor competence during BAT whitening (Huang et al., 2022). Despite these advances, how cellular heterogeneity changes during BAT whitening and which regulatory programs drive this developmental transition remain incompletely understood.

Previous work has shown that BAT deposition in newborn goat kids is most pronounced around the kidneys and that BAT whitening occurs rapidly during the first 28 days after birth, with postnatal day 14 representing a pivotal transition point (Zhao et al., 2022b, 2024). Furthermore, whitening of goat kid PRAT is not driven by brown adipocyte death or white adipocyte proliferation (Zhao et al., 2024). To determine whether the postnatal transition from BAT to WAT-like tissue reflects mature adipocyte transdifferentiation or dynamic remodeling of heterogeneous adipocyte subpopulations with distinct thermogenic and lipid-storage properties, snRNA-seq was performed on goat kid PRAT at postnatal days 0, 7, and 14. This analysis generated a single-nucleus transcriptomic atlas of PRAT, defined cellular subpopulations and developmental trajectories, identified genes and signaling pathways associated with cell-fate regulation, and provided mechanistic insight into the brown-to-white-like adipose transition during early PRAT development in goat kids.

MATERIALS AND METHODS

Animal and sample collection

Male Dazu black goat kids were selected as described

previously (Zhao et al., 2024). All animals were born healthy in January 2022 and were subsequently assigned to three postnatal developmental stages: day 0 (D0, $n=3$), day 7 (D7, $n=3$), and day 14 (D14, $n=3$). The goats were raised under standardized husbandry conditions at the Southwest University Experimental Animal Center, with *ad libitum* access to water and a balanced diet formulated to meet nutritional requirements. Health status was monitored daily by veterinary staff, and no abnormalities were observed throughout the experimental period. Following euthanasia by exsanguination via the jugular vein, PRAT samples were harvested for subsequent analyses. One portion of each PRAT sample was fixed in fixative solution (Servicebio, China), and another portion was immediately frozen in liquid nitrogen and stored at -80°C . All animal procedures adhered to the regulations of the Southwest University Institutional Animal Care and Use Committee (IACUC-20221122-01).

Hematoxylin-eosin (H&E) staining

Fresh PRAT samples were fixed in fixative solution for at least 24 h. Histological processing followed standard protocols (Luo et al., 2021; Zhao et al., 2024). Briefly, samples were dehydrated through a graded ethanol series consisting of 75% (4 h), 85% (2 h), 90% (2 h), 95% (1 h), and 100% (30 min) ethanol, followed by clearing, paraffin wax infiltration, embedding, and sectioning at 5 μm . Tissue sections were subsequently stained with H&E, dehydrated, sealed with neutral gum, and air-dried at room temperature for observation and imaging.

Single-nucleus RNA sequencing

Nuclei were isolated from goat PRAT samples collected at D0, D7, and D14 according to published protocols (Zhang et al., 2024a). Nuclear suspensions were examined microscopically after trypan blue staining to determine total nuclei, nuclear concentration, and the proportion of nuclei with intact membranes. The nuclear concentration was then adjusted to 600–1 200 nuclei/ μL . Barcoded gel beads were added to the nuclear suspension, and the mixture was partitioned in oil to generate Gel Beads-in-Emulsions (GEMs). Sequencing libraries were constructed from GEMs and sequenced on the Illumina platform by Genedenovo Biotechnology Co., Ltd. (China).

Data processing and cell-type annotation

SnRNA-seq data were generated from PRAT samples harvested on D0, D7, and D14. Raw sequencing data were processed using Cell Ranger (<https://support.10xgenomics.com/single-cell-gene-expression/software/overview/welcome>), including filtering, alignment, quantification, and cell identification, to generate gene expression matrices for individual nuclei. Seurat (<https://satijalab.org/seurat/>) was then employed for additional quality filtering, normalization, cell subpopulation classification, differentially expressed gene (DEG) analysis, and marker gene selection. Harmony (Korsunsky et al., 2019) was utilized for batch-effect correction and clustering, followed by calculation of correlations among cell subpopulations. Cells were clustered at a resolution of 0.5, and single-cell subpopulation classifications were visualized using t-distributed stochastic neighbor embedding (t-SNE). Genes up-regulated in more than 25% of cells within a subpopulation, with $P \leq 0.01$ and fold change ≥ 2 , were identified as marker genes. Cell types were manually annotated based on known marker genes from human, mouse, and rabbit datasets and the CellMarker database ([650 www.zoores.ac.cn](http://bio-</p>
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Gene functional analysis

Transcriptional dynamics in adipocyte progenitors, committed preadipocytes, and adipocytes were examined following established procedures (Fan et al., 2023). Briefly, time-series expression profiles of DEGs across consecutive developmental stages were analyzed using the Mfuzz algorithm. DEGs were identified using the Seurat FindMarkers function with thresholds of $\log_2$ fold change > 1 and $P < 0.05$. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were performed using the clusterProfiler v.4.0.2 package (<https://bioconductor.org/packages/clusterProfiler/>) to determine the primary biological functions of DEGs with similar expression patterns.

Pseudotime analysis

Pseudotime analysis was performed with Monocle (Qiu et al., 2017) to identify DEGs associated with distinct adipogenic differentiation trajectories. Monocle2 was then used to reconstruct pseudotime trajectories for adipocyte progenitors, committed preadipocytes, and adipocytes, thereby enabling assessment of developmental progression and cell-state transitions. The “plot pseudotime heatmap” function was applied to create heatmaps depicting the expression patterns of signature genes and highly variable genes across pseudotime.

RNA extraction and quantitative reverse transcription PCR (RT-qPCR)

Total RNA was extracted from D0, D7, and D14 PRAT samples using TRIzol reagent (Invitrogen, USA), according to instructions from the manufacturer (Zhao et al., 2022b, 2024). Reverse transcription was performed with 2 μ L of total RNA using the Prime Script™ RT Reagent Kit with gDNA Eraser (Tiangen, China). qPCR was performed using TB Green® Premix Ex Taq™ II on a real-time PCR instrument (Bio-Rad, USA). All primers were designed with Primer Premier v.5.0 (Premier Biosoft, USA) and are listed in Supplementary Table S1. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method after normalization to goat β -actin as the internal control.

Immunofluorescence

Adipose tissue samples stored at -80°C were flattened with a scalpel and dehydrated in 30% sucrose solution in 50 mL centrifuge tubes. Tissues were then embedded in OCT compound and sectioned into 5–10 μ m cryosections, followed by air drying. Before immunostaining, sections were permeabilized with 0.5% Triton X-100 (Beyotime, China) for 10 min and blocked with 5% bovine serum albumin (BSA) (Beyotime, China). Sections were then incubated overnight at 4°C with primary antibodies (anti-UCP1, 1:200; anti-FABP4, 1:300; anti-CKB, 1:200; anti-PDK4, 1:200; Proteintech, China). After three washes, the sections were incubated with a secondary antibody for 30 min at 37°C and counterstained with 4',6-diamidino-2-phenylindole (DAPI, Beyotime, China). All sections were examined and imaged under an inverted microscope.

Western blotting

Total protein was extracted from PRAT samples collected at D0, D7, and D14 following the manufacturer's protocols (Zhao et al., 2024). Protein samples were loaded at 30 μ g per well and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, USA). Proteins

were then transferred to polyvinylidene fluoride (PVDF) membranes for western blot analysis. After transfer, membranes were blocked in 5% skim milk for 1 h at room temperature and incubated overnight at 4°C with primary antibodies (anti-UCP1, 1:2 000; anti-FABP4, 1:5 000; anti-FASN, 1:5 000; anti-GAPDH, 1:5 000; anti-CKB, 1:2 000; anti-PDK4, 1:2 000; Proteintech, China; and anti-OXPHOS, 1:2 000, Thermo Fisher, USA). After washing, membranes were incubated with secondary antibody (1:1 000, Beyotime, China) for 2 h at room temperature. Protein bands were visualized using Immobilon™ Western Chemiluminescent HRP Substrate (Millipore, USA) and detected with a chemiluminescence imaging system (Bio-Rad, USA).

Statistical analyses

Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism v.9.5 (www.graphpad.com). Multiple comparisons were conducted using one-way analysis of variance (ANOVA). Statistical significance was denoted as: * $P < 0.05$; ** $P < 0.01$.

RESULTS

D14 represents a critical transition point during BAT whitening in Dazu black goats

To confirm D14 as a key stage in BAT whitening in Dazu black goats, PRAT was examined from D0 to D14. PRAT showed a pronounced macroscopic transition from a reddish-brown appearance at D0 to a milky white phenotype at D14, accompanied by enlargement of lipid droplets and acquisition of WAT-like morphology (Figure 1A). Expression of FABP4, FASN, UCP1, and the mitochondrial complex proteins (complexes I–V) was then assessed. FASN expression was significantly higher at D14 than at D0 ($P < 0.01$) and D7 ($P < 0.05$) (Figure 1B, C). In contrast, UCP1 expression was notably reduced at D14 compared with D0 ($P < 0.01$) (Figure 1B, C). Similarly, mitochondrial complex proteins, particularly complexes I, III, and V, were also expressed at significantly higher levels at D0 than at D14 ($P < 0.01$) (Figure 1B, C).

Generation of a developmental-stage-specific single-nucleus atlas of PRAT

Nuclear suspensions were prepared from PRAT samples collected at D0, D7, and D14. Microscopic examination after trypan blue staining revealed minimal nuclear aggregation and clear suspension backgrounds across all samples (Supplementary Figure S1). Nuclei with intact membranes accounted for 90%, 92%, and 92% of suspensions from D0, D7, and D14 PRAT samples, respectively. Nuclear concentrations were 1 215/ μ L, 1 290/ μ L, and 620/ μ L, respectively, and total nuclear counts were 182 250, 129 000, and 31 000, respectively, meeting the requirements for downstream single-nucleus analysis (Supplementary Table S2). Cell-level quality metrics before and after filtering, correlations between gene number and unique molecular identifier (UMI) number, post-quality-control sequencing read numbers from Cell Ranger processing, and reference genome alignment results are shown in Supplementary Figures S2, S3 and Table S3, S4, respectively.

After quality control, t-SNE was used to visualize cell clustering. A total of 17 158, 19 943, and 17 537 cells from D0, D7, and D14 PRAT samples, respectively, were assigned to eight clusters (Figure 2A, B). The relative proportions of these clusters are shown in Supplementary Figure S4. No cluster

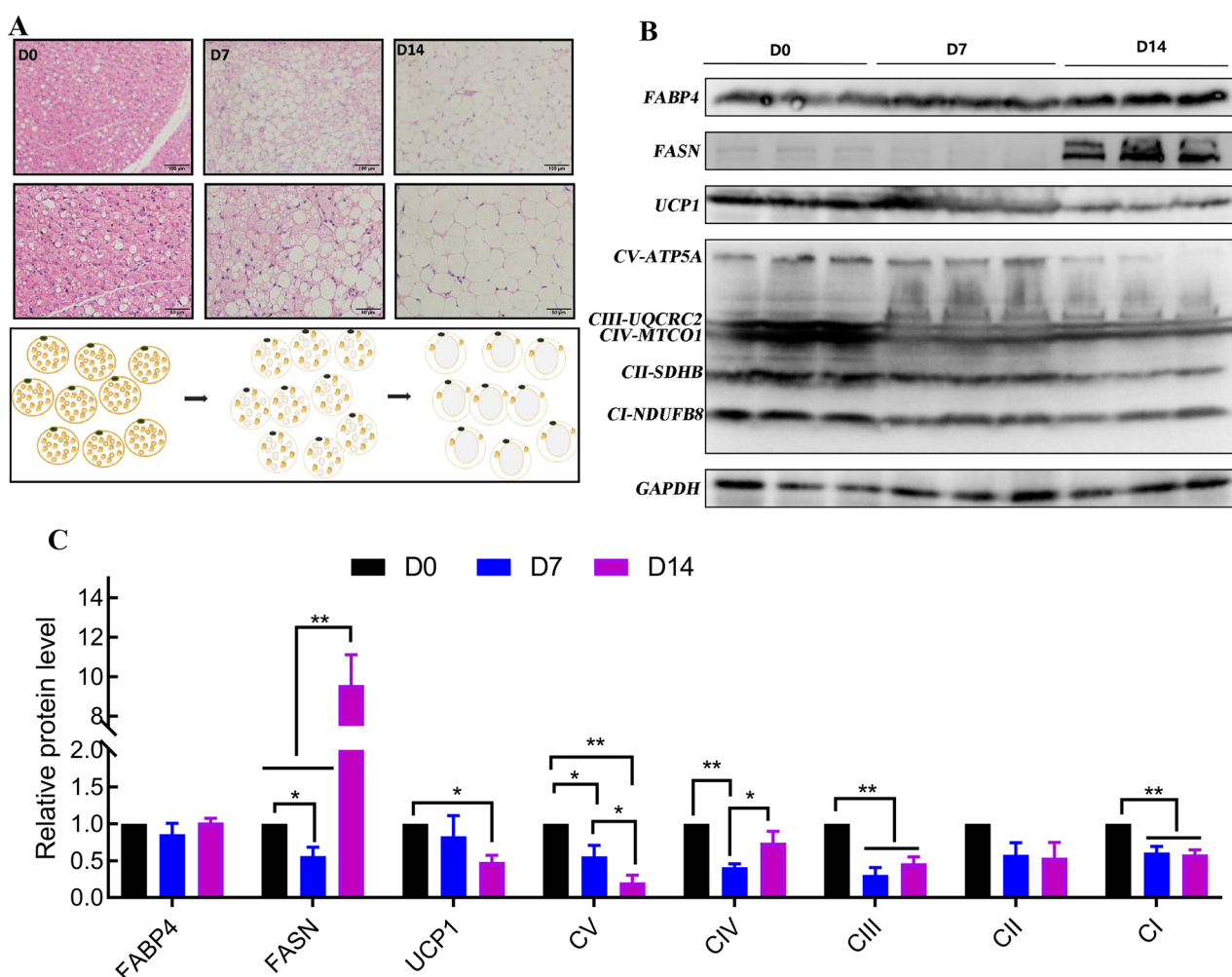


Figure 1 Characteristics of goat kid PRAT

A: H&E staining of PRAT from D0 to D14 ($n=3$). Scale bars: 100 μm (top row) and 50 μm (bottom row). B, C: Western blot analysis of FABP4, FASN, UCP1, and mitochondrial complex proteins ($n=3$). Data are presented as mean $\pm$ SEM. Significance was determined by one-way ANOVA. *: $P<0.05$; **: $P<0.01$, compared with control.

emerged or disappeared between D0 and D14. Pairwise correlation analysis further defined relationships among the eight clusters (Supplementary Figure S5). Cluster 5 and cluster 6 showed a very strong correlation (0.986), suggesting strong transcriptional similarity. High correlations were also detected among clusters 1, 3, 4, and 8, indicating closely related cellular features. In contrast, pairwise correlations between cluster 2 and clusters 3, 5, 6, 7, and 8 were consistently below 0.5, supporting cluster 2 as a distinct cell population. These relationships guided subsequent cluster annotation.

Marker-gene analysis subsequently resolved six major PRAT cell types. Clusters 1, 3, and 4 predominantly expressed adipocyte progenitor marker genes, including *PDGFRA*, *DLK1*, *FGF10*, *SEMA3C*, *CD34*, *FSTL1*, *PDGFRB*, and *CD29* (Figure 2B–D). Cluster 5 expressed markers indicative of committed preadipocytes, including *ABCG1*, *F3*, and *ZNF423* (Figure 2B, E, F). Cluster 6 was characterized by expression of mature adipocyte marker genes, including *PRDM16*, *PPARGC1B*, *PPARGC1A*, *PPARG*, *PNPLA2*, *PLIN1*, *LPL*, *FABP4*, *ELOVL6*, *EBF2*, *CIDEA*, *CD36*, *ADIPOQ*, and *UCP1* (Figure 2B, G, H). Cluster 7 predominantly expressed immune cell marker genes, including *MRC1*, *CD86*, *CD163*, *MAF*, and *CD45* (Supplementary Figure S6). Cluster 8 expressed endothelial cell marker genes, such as *CDH5*,

ENG, *FLT1*, *KDR*, *PECAM1*, and *VWF* (Supplementary Figure S7). Cluster 2 was defined by epithelial cell marker genes associated with kidney tissue, including *ATP1B1*, *KRT8*, *UPK1B*, and *WT1* (Supplementary Figure S8).

Construction of adipocyte lineage differentiation trajectories in goat PRAT

Pseudotime analysis identified one branch point and placed adipocyte progenitors at the trajectory origin, followed by committed preadipocytes and adipocytes (Figure 3A). Pseudotime-dependent genes were classified into five dynamic expression clusters, each showing distinct temporal patterns during lineage progression (Figure 3B). Functional enrichment analysis indicated that adipogenic differentiation was accompanied by activation of metabolic and bioenergetic programs, such as fatty acid metabolism, AMPK signaling, PPAR signaling, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation (Figure 3B). Consistent with lineage progression, adipocyte progenitor marker genes (*PDGFRA*, *FSTL1*, *PDGFRB*, *DLK1*, *FGF10*, and *THY1*) declined along pseudotime, whereas adipocyte marker genes (*UCP1*, *PPARG*, *FABP4*, *LPL*, *PPARGC1A*, and *CD36*) increased (Figure 3C). These expression patterns support the reliability of adipocyte subpopulation annotation and confirm the ordered transition from progenitor states toward mature adipocyte states. Key DEGs associated with the adipogenic

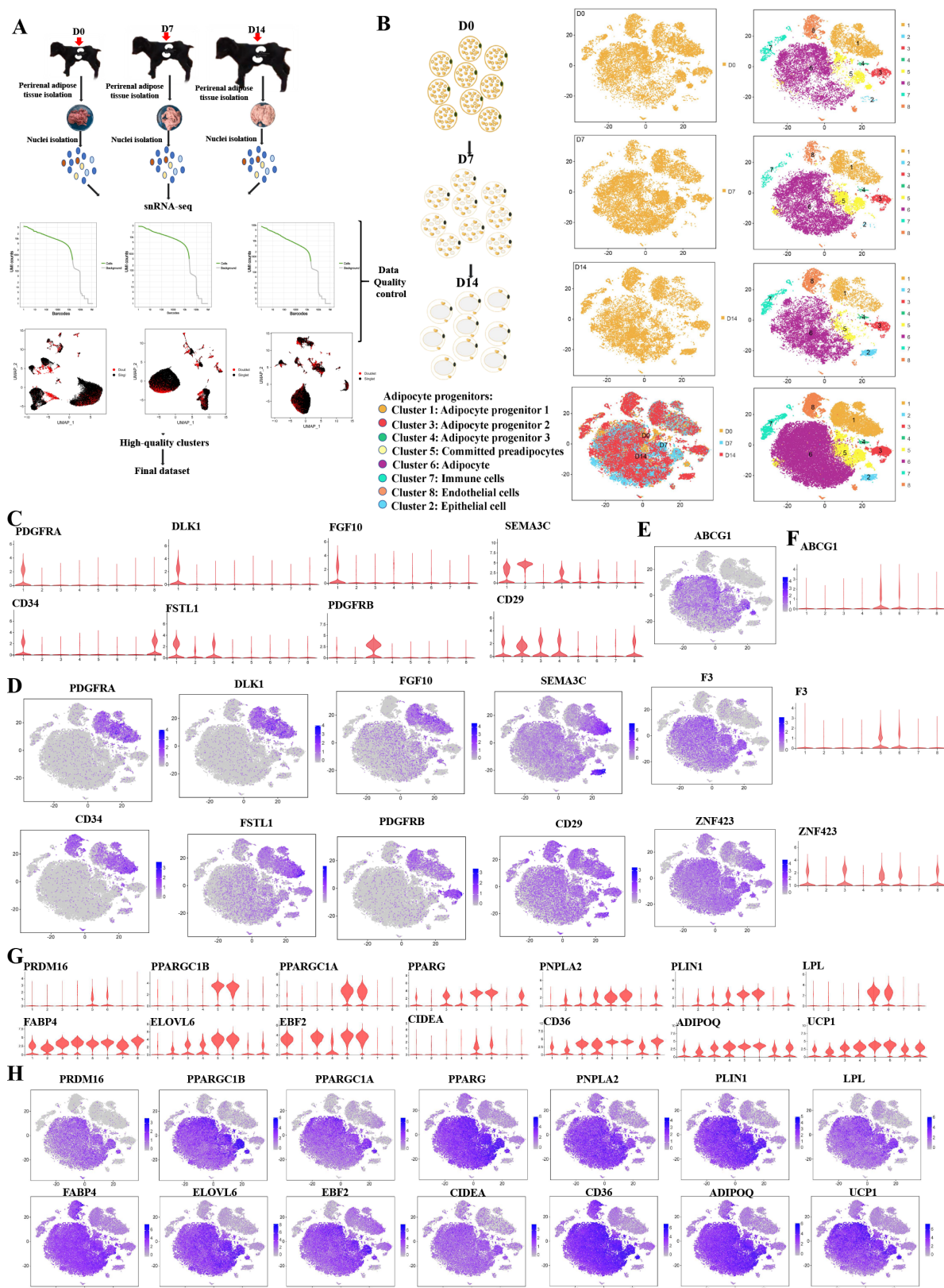


Figure 2 Identification of cell types in goat PRAT

A: Workflow for snRNA-seq analysis of PRAT. B: t-SNE plots showing single-nucleus subpopulation classification in PRAT samples collected at D0, D7, and D14. C: Violin plots showing expression density of adipocyte progenitor marker genes across cell subpopulations. D: t-SNE plots showing expression of adipocyte progenitor marker genes across all cells. E: t-SNE plots showing expression of committed preadipocyte marker genes across all cells. F: Violin plots showing expression density of committed preadipocyte marker genes across cell subpopulations. G: Violin plots showing expression density of adipocyte marker genes across cell subpopulations. H: t-SNE plots showing expression of adipocyte marker genes across all cells.

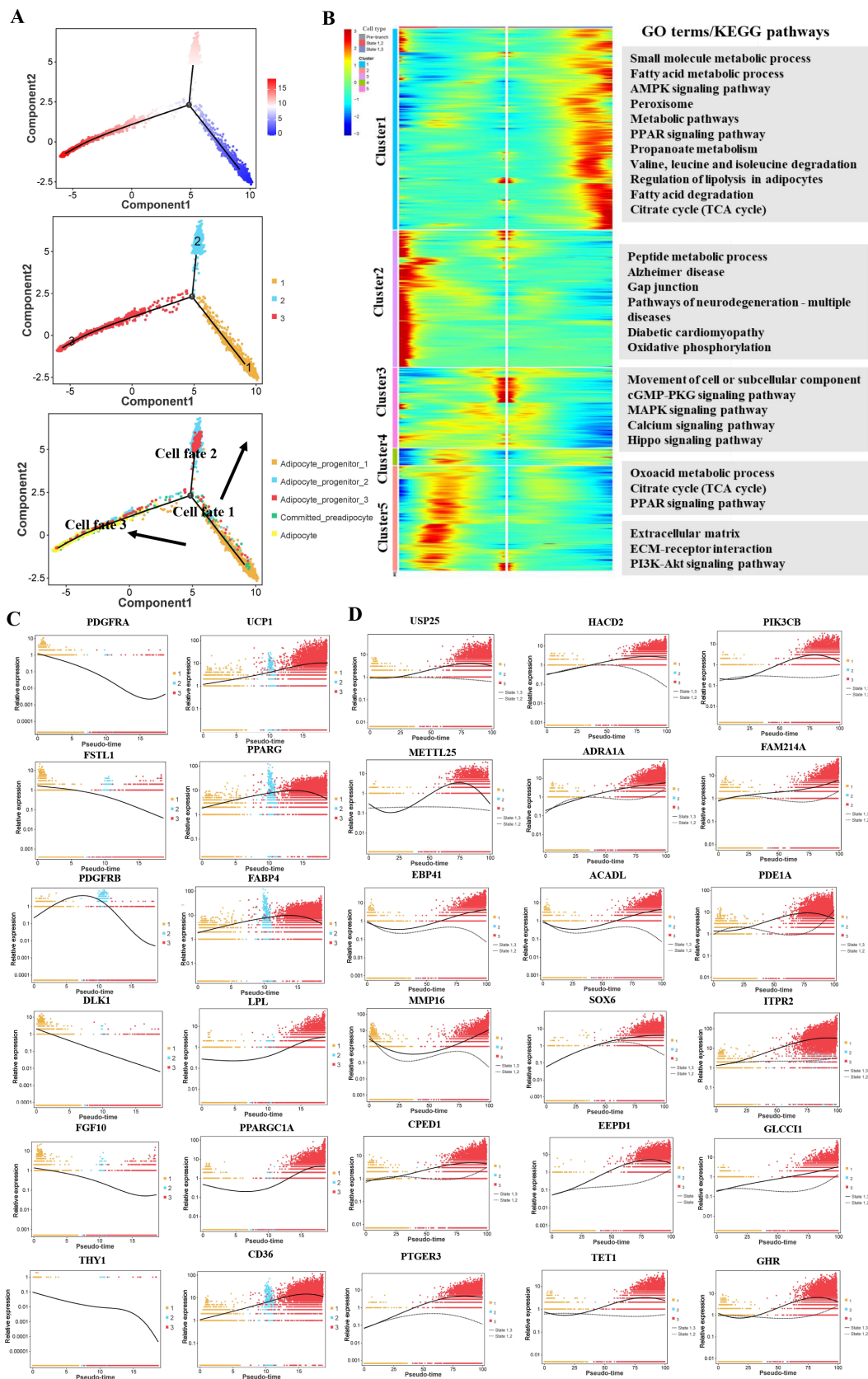


Figure 3 Monocle analysis of adipocyte trajectories in goat PRAT

A: Pseudotime reconstruction of adipocyte lineage differentiation trajectories. B: Heatmap showing gene expression dynamics across adipocyte differentiation fates, with GO and KEGG enrichment analysis for each cluster. C: Pseudotime-dependent expression trends of adipocyte progenitor marker genes (*PDGFRA*, *FSTL1*, *PDGFRB*, *DLK1*, *FGF10*, and *THY1*) and adipocyte marker genes (*UCP1*, *PPARG*, *FABP4*, *LPL*, *PPARGC1A*, and *CD36*). D: Pseudotime-dependent expression trajectories of branching differentially expressed genes, including *USP25*, *HADC2*, *PIK3CB*, *METTL25*, *ADRA1A*, *FAM214A*, *EPB41*, *ACADL*, *PDE1A*, *MMP16*, *SOX6*, *ITPR2*, *CPED1*, *EEPD1*, *GLCC1*, *PTGER3*, *TET1*, and *GHR*.

trajectory, including *USP25*, *HACD2*, *PI3KCB*, *METTL25*, *ADRA1A*, *FAM214A*, *EPB41*, *ACADL*, *PDE1A*, *MMP16*, *SOX6*, *ITPR2*, *CPED1*, *EEPD1*, *GLCCI1*, *PTGER3*, *TET1*, and *GHR*, were further screened and visualized (Figure 3D).

Molecular characterization of adipocyte progenitor subpopulations

As postnatal BAT whitening has been reported in rabbits, humans, and goats (Huang et al., 2022; Zhao et al., 2022b, 2024), shared adipocyte progenitor features across these species were examined. Marker genes from goat adipocyte progenitors in cluster 1 were compared with adipocyte progenitor markers from rabbits and humans. This analysis identified 74 overlapping genes between humans and goats, 47 overlapping genes between rabbits and goats, and 50 overlapping genes between humans and rabbits (Figure 4A; Supplementary Table S5). Moreover, 15 genes were shared among all three species: *FSTL1*, *MAP1B*, *DPT*, *TGFBR2*, *ANTXR1*, *PCSK5*, *DLK1*, *SEMA3C*, *DPYSL3*, *COLEC12*, *COL12A1*, *AHNAK*, *FBLN5*, *ASPN*, and *MFAP5* (Figure 4A; Supplementary Table S5). Among these shared markers, nine encoded extracellular matrix proteins, indicating a prominent extracellular matrix-associated and secretory signature in adipocyte progenitors. Visualization of these 15 shared genes on the t-SNE plot showed that *DPT*, *DLK1*, and *MFAP5* were specifically expressed in cluster 1 (Figure 4B).

To define transcriptional dynamics in adipocyte progenitors across adjacent developmental stages, DEGs were identified using the Seurat FindMarkers function and analyzed by fuzzy clustering. Adipocyte progenitors clustered into three time-dependent transcriptional patterns (Figure 5A). Cluster 1 contained genes preferentially expressed at D0, with enrichment in peptide metabolism, protein metabolism, and oxidative phosphorylation (Figure 5A). Cluster 3 contained genes highly expressed at D7, with enrichment in biological process regulation, PI3K-Akt signaling, and mTOR signaling (Figure 5A). Cluster 2 contained genes preferentially expressed at D14, with enrichment in lipid metabolism, PPAR signaling, AMPK signaling, adipocytokine signaling, FoxO

signaling, insulin signaling, PI3K-Akt signaling, and fatty acid metabolism, indicating activation of lipid-synthesis-related programs at this stage (Figure 5A).

Consistent with this developmental shift, adipogenesis-related genes, including *DGAT2*, *CD36*, *FABP4*, *PDK4*, *PPARG*, and *PLIN1*, increased progressively from D0 to D14 (Figure 5B), suggesting enhanced lipogenic differentiation potential in adipocyte progenitors during postnatal PRAT remodeling. To determine whether this pattern reflected heterogeneity within the adipocyte progenitor compartment, progenitor cells were further resolved into seven subclusters. The top five DEGs for each subcluster were mapped to define subcluster-specific transcriptional features (Figure 5C, E). These genes represent candidate markers for distinguishing adipocyte progenitor subtypes in goat PRAT. Interestingly, among the seven subclusters, clusters 0, 1, and 2 showed the strongest developmental-stage bias (Figure 5D). Further analysis of adipogenesis-related genes demonstrated high expression of *DGAT2*, *CD36*, *FABP4*, *PDK4*, *PPARG*, and *PLIN1* in cluster 0, which was predominantly detected at D14 (Figure 5F).

Molecular characterization of committed preadipocyte subpopulations

To determine whether committed preadipocytes acquired a progressively stronger lipogenic program during postnatal PRAT remodeling, stage-dependent transcriptional changes were analyzed from D0 to D14. Fuzzy clustering resolved four time-dependent expression programs in committed preadipocytes. Genes highly expressed at D0 (cluster 2) were primarily enriched in mitochondrial processes, oxidative phosphorylation, thermogenesis, and the TCA cycle (Figure 6A). At D7, highly expressed genes (cluster 1) were mainly enriched in cellular metabolic processes, ubiquitin-mediated proteolysis, autophagy, and inositol phosphate metabolism (Figure 6A). By D14, highly expressed genes (cluster 4) were primarily enriched in regulation of cellular metabolic processes, AMPK signaling, PPAR signaling, and PI3K-Akt signaling (Figure 6A). These findings indicate that

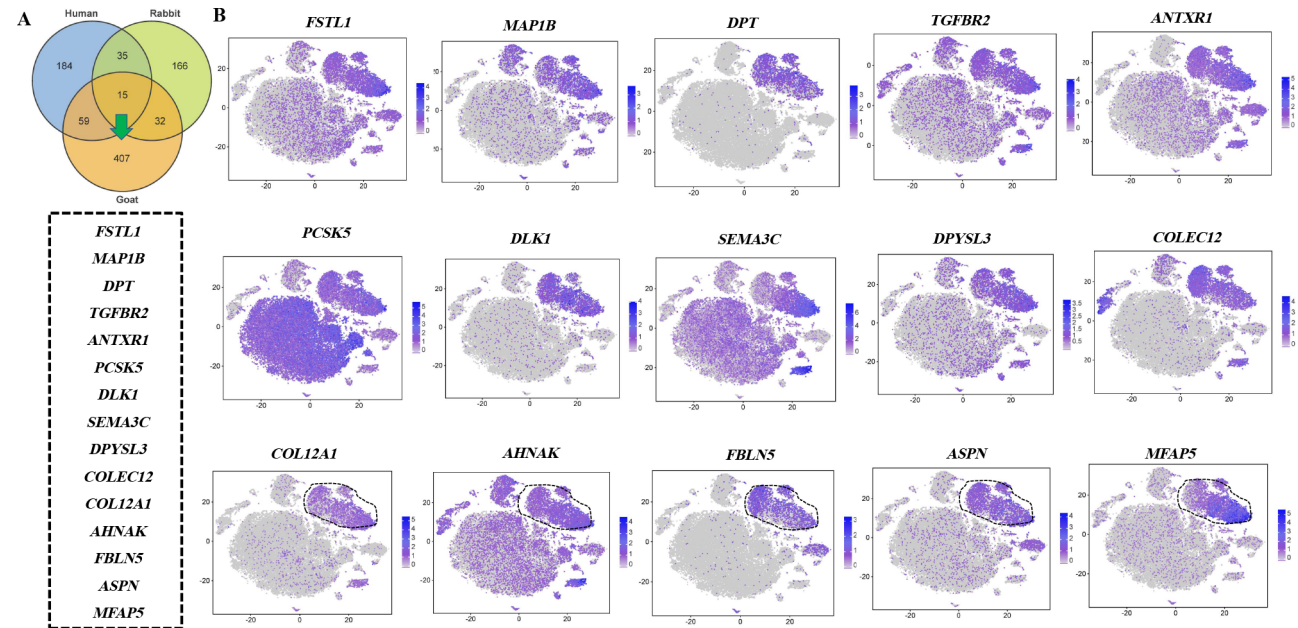


Figure 4 Conservation analysis of adipocyte progenitors in goat PRAT

A: Venn diagram showing marker genes shared among goat, rabbit, and human adipocyte progenitors. B: t-SNE plots showing the distribution of conserved brown adipocyte progenitor marker genes shared by goats, rabbits, and humans.

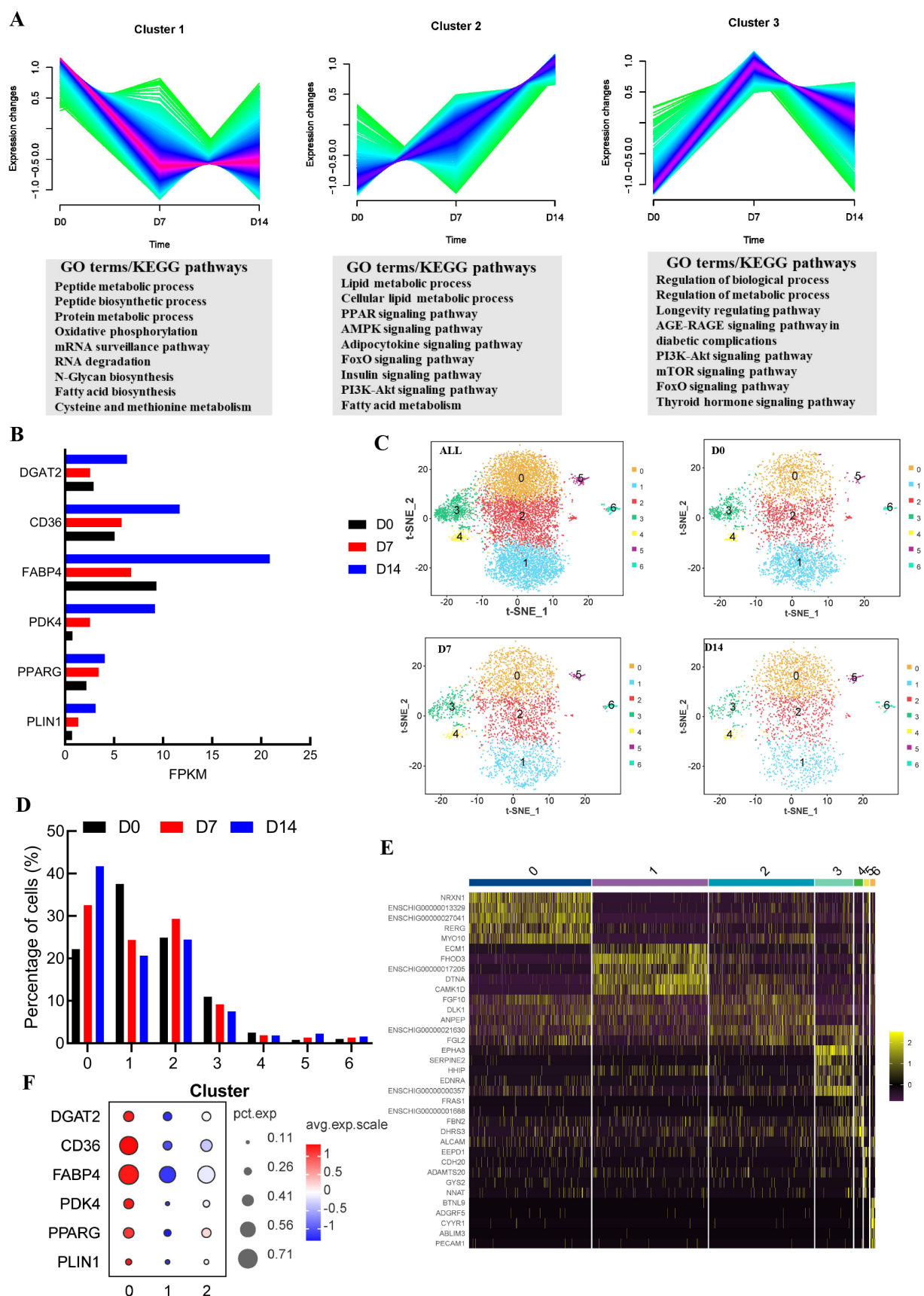


Figure 5 Molecular characterization of adipocyte progenitor subpopulations

A: Fuzzy clustering of adipocyte progenitors across developmental stages (D0, D7, and D14), with GO and KEGG enrichment analysis for each cluster. Yellow and green lines correspond to genes with low membership values, and red and purple lines correspond to genes with high membership values. B: Expression levels of lipid deposition-associated genes. C: Subclustering of adipocyte progenitors. D: Cell proportions across adipocyte progenitor subclusters. E: Heatmap showing differentially expressed genes across adipocyte progenitor subclusters. F: Bubble plots showing expression of lipid deposition-associated genes across adipocyte progenitor subclusters. Pct. exp, percentage of expressed cells.

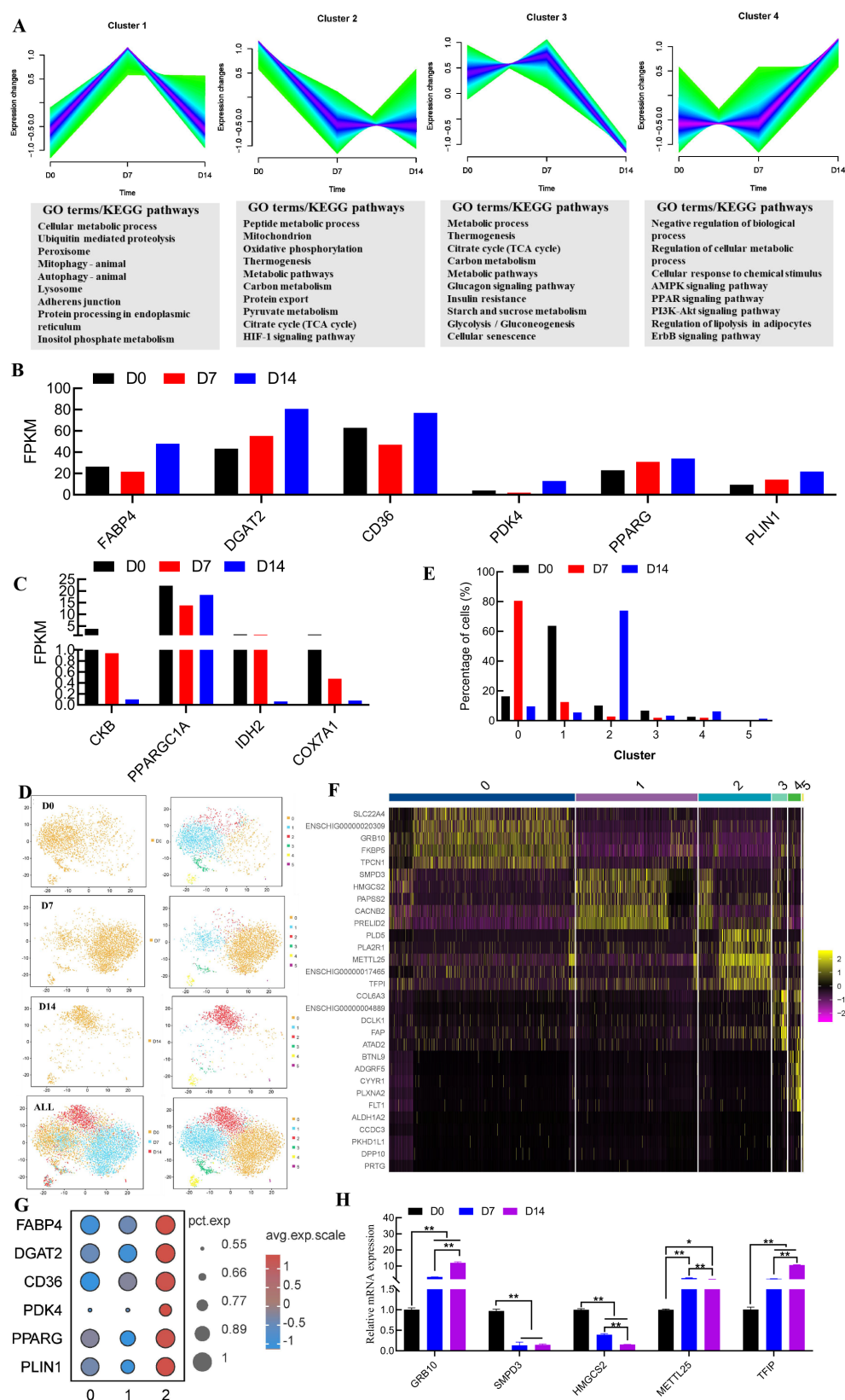


Figure 6 Molecular characterization of committed preadipocyte subpopulations

A: Fuzzy clustering of committed preadipocytes across developmental stages (D0, D7, and D14), with GO and KEGG enrichment analysis for each cluster. Yellow and green lines correspond to genes with low membership values, and red and purple lines correspond to genes with high membership values. B: Expression levels of lipid deposition-associated genes. C: Expression levels of thermogenesis-related genes. D: Subclustering of committed preadipocytes. E: Cell proportions across committed preadipocyte subclusters. F: Heatmap of differentially expressed genes across committed preadipocyte subclusters. G: Bubble plots showing expression of lipid deposition-associated genes across committed preadipocyte subclusters. H: qPCR validation of snRNA-seq data reliability ($n=3$). Data are presented as mean $\pm$ SEM. Significance was determined by one-way ANOVA. *: $P<0.05$; **: $P<0.01$, compared with control. Pct. exp, percentage of expressed cells.

committed preadipocytes underwent a developmental transition from thermogenic and oxidative programs toward signaling states associated with lipid metabolism and adipogenic maturation. Consistent with this shift, adipogenesis-related genes, including *DGAT2*, *CD36*, *FABP4*, *PDK4*, *PPARG*, and *PLIN1*, increased over time (Figure 6B), whereas thermogenesis-related genes, including *CKB*, *PPARGC1A*, *IDH2*, and *COX7A1*, declined (Figure 6C).

To determine whether this transcriptional shift reflected heterogeneity within the committed preadipocyte compartment, committed preadipocytes were further divided into six subclusters. The top five DEGs for each subcluster were mapped to define subcluster-specific transcriptional features (Figure 6D, F). These genes represent potential markers for distinguishing committed preadipocyte subtypes in goat PRAT. Among the six subclusters, clusters 0, 1, and 2 exhibited distinct developmental timing characteristics (Figure 6E). Further analysis of adipogenesis-related genes demonstrated high expression of *DGAT2*, *CD36*, *FABP4*, *PDK4*, *PPARG*, and *PLIN1* in cluster 2, which was predominantly detected at D14 (Figure 6G). To validate the snRNA-seq results, five genes highly expressed in committed preadipocytes (*GRB10*, *SMPD3*, *HMGCS2*, *METTL25*, and *TFIP*) were selected for qPCR analysis (Figure 6H). The qPCR results showed a strong correlation with the snRNA-seq data, confirming the reliability of the transcriptomic profiles.

Together, these results showed that committed preadipocytes underwent coordinated transcriptional remodeling during early postnatal development, with attenuation of thermogenesis-related programs and activation of adipogenic and lipid-metabolic pathways. AMPK and PPAR signaling at D14 were closely associated with this lipogenic transition. The shift was further explained by expansion of a committed preadipocyte subpopulation linked to lipid deposition and reduction of subpopulations associated with thermogenic adipocyte features.

Molecular characterization of adipocyte subpopulations

Given the progressive activation of lipogenic programs in adipocyte progenitors and committed preadipocytes, adipocytes were next analyzed to determine whether mature adipocyte states underwent similar developmental remodeling. Fuzzy clustering of stage-dependent gene expression resolved six time-dependent expression patterns in adipocytes (Figure 7A). At D0, highly expressed genes (clusters 1 and 3) were primarily enriched in thermogenesis, oxidative phosphorylation, the TCA cycle, and insulin signaling (Figure 7A). At D7, highly expressed genes (clusters 2 and 5) were mainly enriched in oxidative phosphorylation, thermogenesis, AMPK signaling, and PPAR signaling (Figure 7A). At D14, highly expressed genes (clusters 4 and 6) were primarily enriched in fatty acid metabolism, AMPK signaling, PPAR signaling, and PI3K-Akt signaling (Figure 7A). Consistent with this trajectory, adipogenesis-related genes, including *DGAT2*, *CD36*, *FABP4*, *PDK4*, *PPARG*, and *PLIN1*, increased along the developmental axis (Figure 7B), whereas thermogenesis-related genes, including *CKB*, *PPARGC1A*, *IDH2*, and *COX7A1*, declined (Figure 7C).

To determine whether this transition reflected adipocyte heterogeneity, mature adipocytes were further resolved into eight subclusters. The top five DEGs for each subcluster were mapped to define subcluster-specific transcriptional features (Figure 7D, F). These genes represent potential markers for identifying adipocyte subtypes in goat PRAT. Among the eight

adipocyte subclusters, clusters 0, 1, 2, and 3 exhibited the strongest developmental timing characteristics (Figure 7E). Adipogenesis-related genes were highly expressed in cluster 2, which was predominantly detected at D14 (Figure 7G). In contrast, thermogenesis-related genes were highly expressed in cluster 0 (Figure 7H). The reliability of the adipocyte snRNA-seq profiles was further supported by qPCR, immunofluorescence, and western blot analyses (Figure 8A–D).

These findings indicate that postnatal adipocyte remodeling involved a coordinated shift in AMPK- and PPAR-associated transcriptional programs toward enhanced lipid biosynthesis and storage. This transition reflected a redistribution of adipocyte states, with progressive enrichment of lipid deposition-associated subpopulations and concomitant depletion of thermogenesis-associated subpopulations.

DISCUSSION

BAT-dependent thermogenesis declines with age in humans, rabbits, sheep, and goats, accompanied by progressive morphological convergence between BAT and WAT adipocytes (Basse et al., 2015; Heaton, 1972; Huang et al., 2022; Kotzbeck et al., 2018; Shimizu et al., 2014; Tanuma et al., 1975; Zhao et al., 2022b, 2024). The regulatory mechanisms that drive BAT involution remain largely unknown, particularly in large mammals such as goats and humans. Molecular strategies that prevent or delay BAT involution may therefore have potential relevance for metabolic disease intervention and for improving neonatal survival in livestock (Huang et al., 2022; Zhao et al., 2024). Single-cell transcriptomic studies of adipose tissue from mice, humans, rabbits, and other species have revealed extensive cellular heterogeneity within adipose tissue (Emont et al., 2022; Huang et al., 2022). Previous research has also suggested that BAT whitening in goat kid PRAT is not caused by brown adipocyte death or white adipocyte proliferation (Zhao et al., 2024). To clarify the cellular and molecular basis of the BAT-to-WAT-like transition during goat PRAT development, this study generated a comprehensive single-nucleus transcriptomic atlas of goat PRAT. The resulting data provide new insight into the cell-state remodeling and transcriptional programs associated with BAT whitening in early postnatal goats.

Histological analysis showed a rapid transition from BAT to WAT-like morphology in Dazu black goat PRAT between birth and D14, identifying D14 as a critical stage in this remodeling process. This pattern is consistent with findings in sheep PRAT, in which white-like adipocytes became predominant from day 14 (Basse et al., 2015), and further corroborates earlier observations in goat kids (Zhao et al., 2024). This study focused on three specific time points (D0, D7, and D14) based on preliminary data and previous reports, which indicated that these stages capture critical events in early adipose tissue development. This design enabled resolution of key cellular heterogeneity by single-nucleus analysis, but it may not fully capture finer temporal dynamics during the transition from thermogenic to lipogenic phenotypes. Future studies incorporating additional intermediate time points, including cost-effective bulk RNA-seq for broader temporal profiling, could refine understanding of stage-specific molecular and cellular changes during PRAT remodeling. From a husbandry perspective, these findings highlight the importance of thermal management during the first 14 days after birth. Exposure to

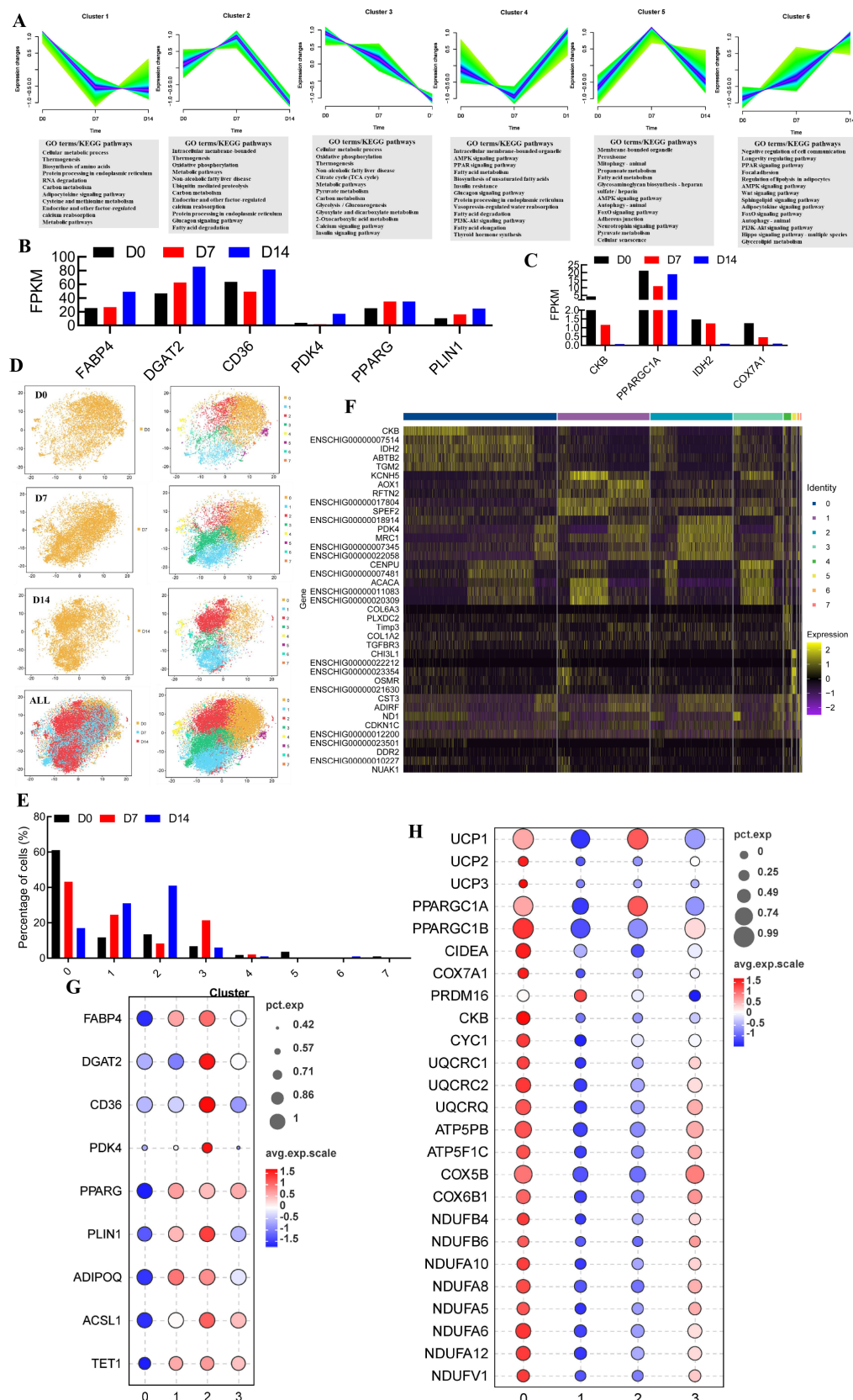


Figure 7 Molecular characterization of adipocyte subpopulations

A: Fuzzy clustering of adipocytes across developmental stages (D0, D7, and D14), with GO and KEGG enrichment analysis for each cluster. Yellow and green lines correspond to genes with low membership values, and red and purple lines correspond to genes with high membership values. B: Expression levels of lipid deposition-associated genes. C: Expression levels of thermogenesis-related genes. D: Subclustering of adipocytes. E: Cell proportions across adipocyte subclusters. F: Heatmap of differentially expressed genes across adipocyte subclusters. G: Bubble plots showing expression of lipid deposition-associated genes across adipocyte subclusters. H: Bubble plots showing expression of thermogenesis-related genes across adipocyte subclusters. Pct. exp, percentage of expressed cells.

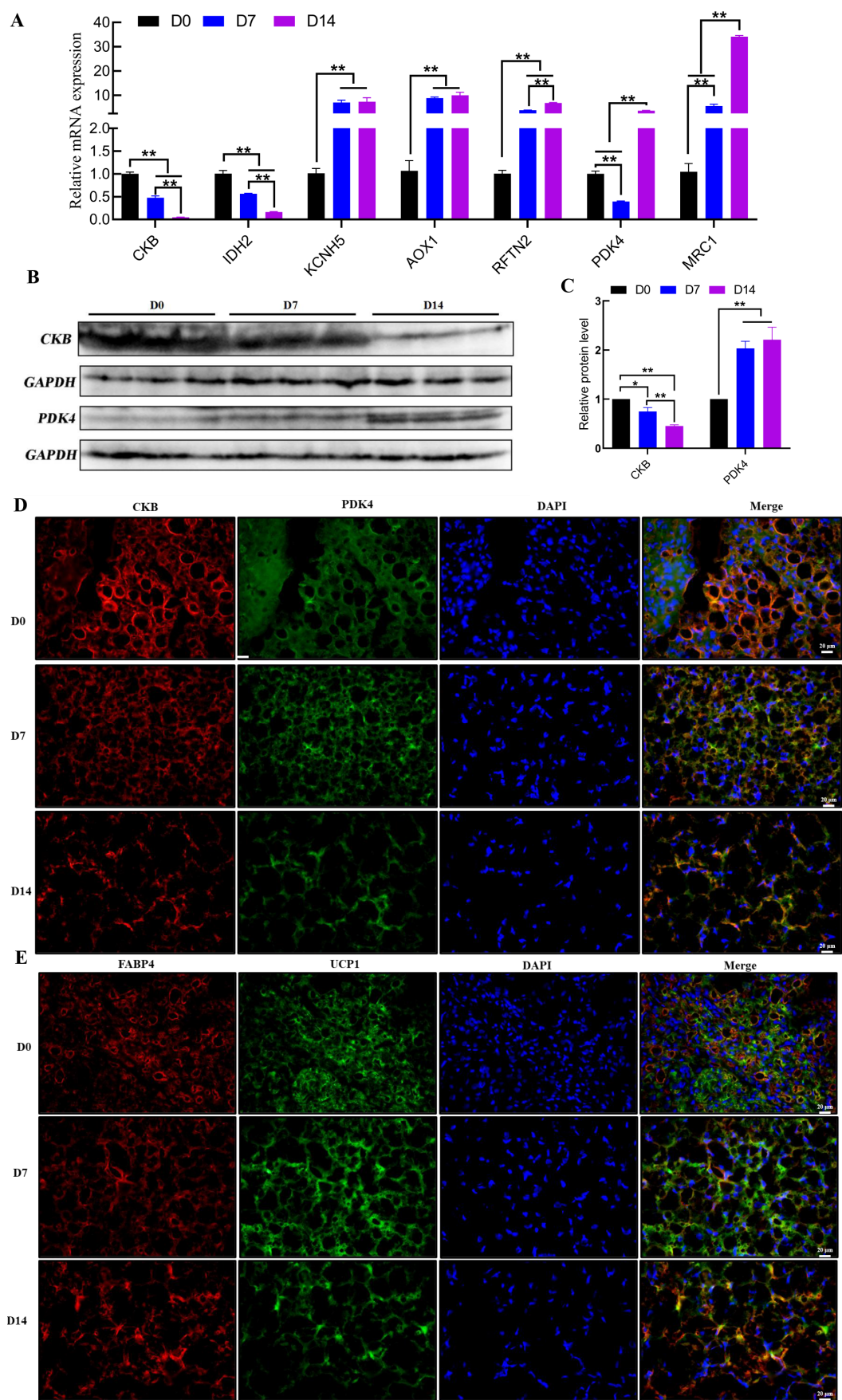


Figure 8 Validation of snRNA-seq-identified differentially expressed genes

A: qPCR validation of snRNA-seq data reliability ($n=3$). B, C: Western blot analysis of CKB and PDK4 protein expression ($n=3$). D, E: Immunofluorescence staining of CKB and PDK4 proteins ($n=3$). Data are presented as mean $\pm$ SEM. Significance was determined by one-way ANOVA. *: $P<0.05$; **: $P<0.01$, compared with control.

low temperatures and large temperature fluctuations in goat kid housing should be minimized (Bélanger-Naud et al., 2021), and feed and water temperatures should be maintained within an appropriate range (Coloma-García et al., 2020). Such measures may help support BAT-dependent thermoregulation and reduce cold stress during the early neonatal period (McKnight et al., 2020).

Marker genes including *PDGFRA*, *PDGFRB*, *CD34*, *DLK1*, *FSTL1*, *CD29*, and *SEMA3C* have previously been identified as markers of brown adipocyte progenitors (Huang et al., 2022; Shamsi et al., 2021; Sun et al., 2020). Using snRNA-seq, Huang et al. (2022) identified one adipocyte progenitor population in human BAT (group 0) and three adipocyte progenitor populations in rabbit BAT (groups 0, 2, and 3). Comparable progenitor populations were identified in goat PRAT in the current study. Specifically, the human brown adipocyte progenitor population defined by *DLK1*, *THY1*, *FSTL1*, and *CD34* expression and the rabbit adipocyte progenitor populations marked by *DLK1* and *FSTL1* expression showed transcriptional similarity to goat adipocyte progenitor clusters 1, 3, and 4. Schwalie et al. (2018) identified three cellular subpopulations, P1, P2, and P3, with the *F3* (*CD142*)- and *ABCG1*-expressing P3 population classified as preadipocytes and showing high similarity to the preadipocytes identified in goat PRAT cluster 5. In mouse BAT, Song et al. (2020) resolved brown adipocytes into low-thermogenic (BA-L) and high-thermogenic (BA-H) subpopulations, with BA-H characterized by high expression of *UCP1*, *ELOVL6*, *CIDEA*, and *PNPLA2*. These brown adipocyte states showed dynamic interconversion in response to changes in environmental temperature.

Further snRNA-seq analysis of mouse BAT under room temperature (22°C), thermoneutral (30°C), and cold (8°C) conditions identified 10 brown adipocyte subpopulations (P1–P10), with cell-state composition strongly associated with ambient temperature. P1 and P2 were predominant at room temperature and after cold exposure, P3 was enriched under thermoneutral conditions, P6–P10 appeared after cold exposure, and P4 was detected across all temperature conditions. The P4 subpopulation represented mature brown adipocytes with high expression of *UCP1*, *CIDEA*, *PPARGC1A*, *ADIPOQ*, and *PLIN1* (Sun et al., 2020), consistent with the mature adipocyte cluster identified in goat PRAT (cluster 6). Endothelial populations identified in previous BAT and adipose tissue studies also showed strong correspondence with goat PRAT annotations. Shamsi et al. (2021) defined endothelial cells in BAT based on *CDH5*, *PECAM1*, *ICAM2*, and *KDR* marker genes, while Vijay et al. (2020) identified three endothelial cell types (E1, E2, and E3) in adipose tissue, with E2 predominantly expressing classical endothelial markers such as *ACKR1*, *SELE*, *TM4SF1*, *VCAM1*, *TMEM173*, *PLVAP*, *ICAM1*, *PECAM1*, *VWF*, *ADAMTS9*, and *TFPI*. These profiles align with the endothelial cluster identified in goat PRAT (cluster 8), which expressed *CDH5*, *ENG*, *FLT1*, *KDR*, *PECAM1*, and *VWF*. Notably, *VWF* is widely used as an endothelial cell marker (Leng et al., 2024). Immune cell markers, including *CD163*, *CD206* (*MRC1*), and *PTPRC* (*CD45*), have also been reported previously (Hildreth et al., 2021), consistent with the immune cell cluster identified in this study (cluster 7).

BAT involution has been reported during postnatal development in rabbits (Huang et al., 2022), humans (Schosserer et al., 2018), and goats (Zhao et al., 2024). Comparative analysis of adipocyte progenitors in rabbits and

humans previously identified 50 shared progenitor-associated genes (Huang et al., 2022). Extending this cross-species framework, the present study compared adipocyte progenitors from goats, rabbits, and humans and identified cluster 1 as the most abundant progenitor cluster in goat PRAT, representing an early-stage adipocyte progenitor population. Comparative marker-gene analysis detected 74 overlapping genes between human and goat adipocyte progenitors and 47 overlapping genes between rabbit and goat adipocyte progenitors. Moreover, 15 marker genes were shared across adipocyte progenitors from all three species, with nine of these marker genes encoding extracellular matrix proteins, consistent with previous evidence that adipocyte progenitors show prominent extracellular matrix-associated signatures (Huang et al., 2022).

Previous work proposed three potential mechanisms underlying BAT involution and excluded the first hypothesis as the principal cause of this process in goat PRAT (Zhao et al., 2024). In the present study, pseudotime reconstruction, clustering, and subpopulation analysis of adipocyte progenitors, committed preadipocytes, and mature adipocytes indicated progressive enhancement of lipid-synthesis-associated programs during postnatal development. This transition appeared to reflect expansion of subpopulations associated with adipose deposition within each adipocyte-lineage compartment. Lipid metabolism (Ma et al., 2023), fatty acid metabolism (Morigny et al., 2021), AMPK signaling (Göransson et al., 2023), and PPAR signaling (Katzer et al., 2021) have been reported to participate in the processes of fat synthesis, metabolic regulation, and adipose deposition, supporting the biological relevance of these transcriptional changes. Epigenetic regulation, including DNA methylation and histone modifications, along with post-transcriptional mechanisms such as miRNAs and RNA-binding proteins, may also contribute to adipocyte fate determination and modulation of the transition between thermogenic and lipogenic brown adipocyte phenotypes, and these mechanisms warrant further investigation (Chaturvedi et al., 2025). In addition, goats represent a ruminant model with distinct adipose remodeling patterns compared to humans and rodents. Rumen microbiota-derived short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, can regulate fat metabolism and adipose tissue development in ruminants (Pan et al., 2025; Zhang et al., 2024b). These species-specific metabolic features emphasize the necessity of considering our findings within the broader mammalian metabolic framework while also accounting for physiological and environmental adaptations specific to ruminants. Taken together, these findings suggest the coexistence of thermogenesis-enriched and lipid-deposition-enriched brown adipocyte states in PRAT of goat kids, with thermogenesis-enriched subpopulations predominating at birth and lipid-deposition-associated subpopulations becoming more prominent during subsequent postnatal development. However, cold-exposure experiments will be required to determine whether lipid-deposition-enriched brown adipocyte subpopulations can restore *UCP1* expression and reacquire a thermogenic phenotype. Future studies should also examine interactions among rumen microbiota, SCFAs, and adipose tissue remodeling in goats, together with the roles of epigenetic and post-transcriptional regulatory mechanisms in these processes.

In conclusion, this study generated a comprehensive single-nucleus transcriptomic atlas of goat PRAT (Figure 9), resolved

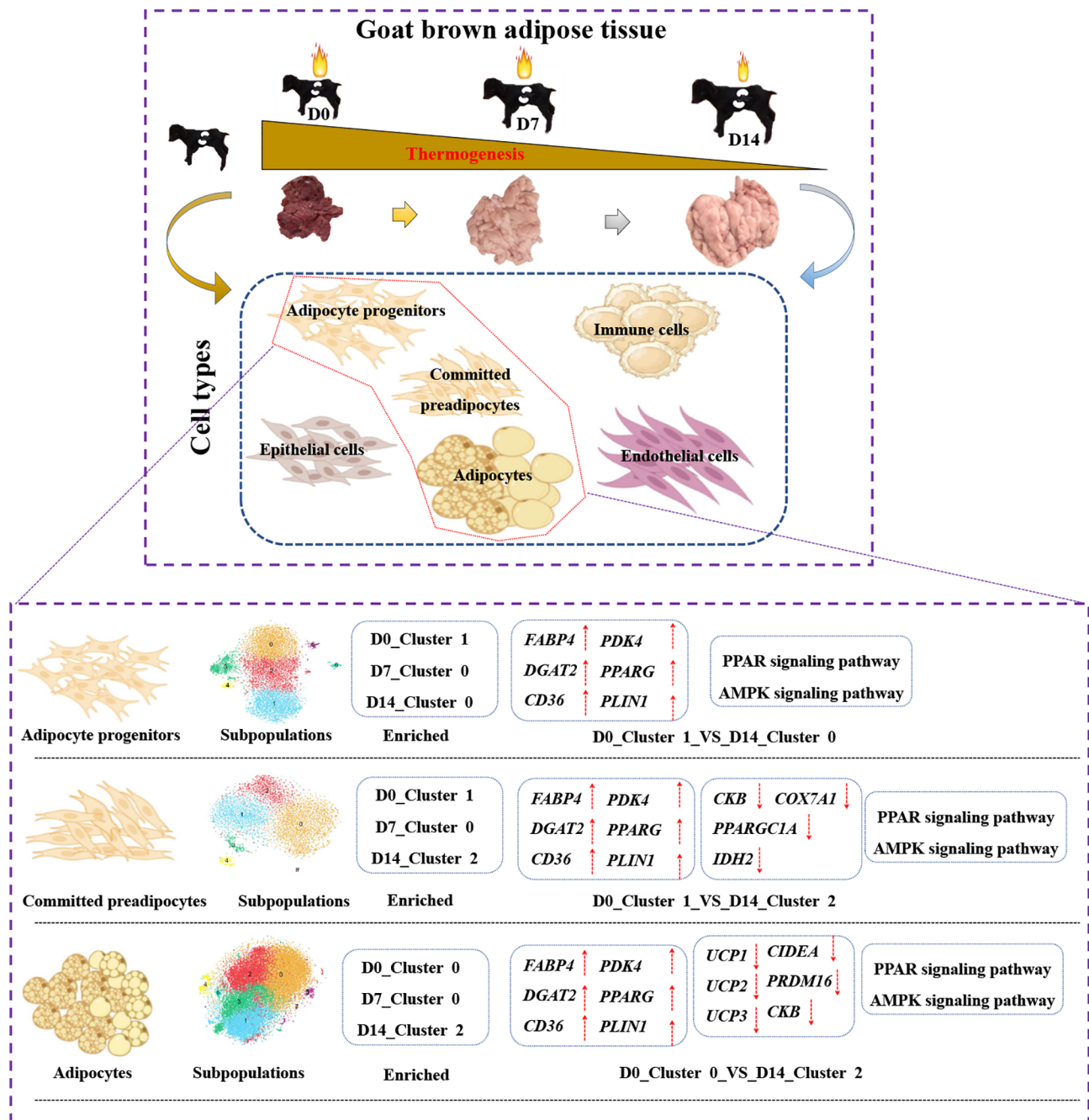


Figure 9 Study summary

D14 was identified as a critical stage during BAT whitening in Dazu black goats. Goat kid PRAT showed marked cellular heterogeneity and contained six distinct cell types, including adipocyte progenitors, committed preadipocytes, and mature adipocytes. This study also revealed the coexistence of thermogenesis-enriched and lipid-deposition-enriched brown adipocyte states, with thermogenesis-enriched subpopulations prevailing at D0, followed by a shift to lipid-deposition-associated subpopulations.

cellular heterogeneity within this depot, and characterized adipocyte progenitors, committed preadipocytes, and mature adipocytes across an early postnatal developmental trajectory. A central finding was the coexistence of thermogenesis-enriched and lipid-deposition-enriched brown adipocytes, with the high-thermogenic subpopulations prevailing at D0, followed by a shift to subpopulations associated with high-fat deposition by D14. This change indicates that adipocyte progenitors, committed preadipocytes, and mature adipocytes progressively adopt transcriptional programs favoring lipid deposition, providing a cellular and molecular basis for the BAT-to-WAT-like transition within the first 14 days after birth.

DATA AVAILABILITY

Datasets generated in this study were deposited in the National Center for Biotechnology Information (NCBI) database (<https://submit.ncbi.nlm.nih.gov/>) (SUB16129445, BioProjectID PRJNA1456385), Genome Sequence Archive (GSA) database (<https://ngdc.cncb.ac.cn/gsa/>) (subPRO063491, PRJCA043182), and Science Data Bank (ScienceDB) (<https://doi.org/10.57760/sciencedb.j00139.00282>).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.Z. Writing–review & editing, Writing–original draft, Validation, Resources, Methodology, Formal analysis, Data curation, Conceptualization. Q.J.Z. Writing–original draft, Validation, Resources, Methodology, Formal analysis, Data curation, Conceptualization. M.H.L. Data curation, Conceptualization. H.L.Y. Writing–review & editing, Methodology, Conceptualization. Y.J.Z. Writing–review & editing, Methodology, Funding acquisition, Conceptualization. All authors read and approved the final version of the manuscript.

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