

Idh2- α -KG axis dysregulation disrupts epigenetic landscape and imprinted genes, inducing placental hypertrophy in SCNT *Bos taurus*

Xiao-Yu Zhao ^{1,2,#}, Yuan Yun ^{1,2,#}, Hong-Yi Liao ^{1,2}, Shao-Ying Tao ^{1,2}, Shan-Shan Wu ^{1,2}, Yu-Xin Gao ^{1,2}, Chun-Jie Bo ^{1,2}, Lei Yang ^{1,2}, Xue-Fei Liu ^{1,2}, Li-Shuang Song ^{1,2}, Chun-Ling Bai ^{1,2}, Guang-Peng Li ^{1,2,*}, Guang-Hua Su ^{1,2,*}

¹State Key Laboratory of Reproductive Regulation and Breeding of Grassland Livestock (R2BGL), Inner Mongolia University, Hohhot, Inner Mongolia 010070, China

²College of Life Sciences, Inner Mongolia University, Hohhot, Inner Mongolia 010070, China

*Corresponding authors. E-mails: gpengli@imu.edu.cn; guanghuasu@imu.edu.cn

#These authors contributed equally to this work.

ABSTRACT

Placental hypertrophy is a major cause of pregnancy failure in somatic cell nuclear transfer (SCNT) animals, with metabolic-epigenetic crosstalk proposed as a key regulatory node, yet its molecular mechanism remains elusive. Isocitrate dehydrogenase 2 (Idh2) is a critical mitochondrial metabolic enzyme that controls the production of α -Ketoglutarate (α -KG), a central regulator of epigenetic modifications. In SCNT *Bos taurus* placentomes and derived placental mesenchymal cells, this study found abnormally elevated α -KG levels. Further functional experiments in these cells suggested that elevated α -KG levels were associated with significant increases in 5-hydroxymethylcytosine (5hmC) and the active histone marker H3K4me3, as well as

decreases in the repressive markers H3K9me3 and H3K27me3, accompanied by altered expression of ten-eleven translocation (TET) dioxygenases and lysine demethylase (KDMs). This ultimately led to the dysregulation of key imprinted genes associated with placental development, including upregulation of *Igf2r* and *Znf597*, and downregulation of *Phlda2*, *Peg10*, and *Magel2*. Further analysis revealed that *Idh2*, a key enzyme for α -KG synthesis, was significantly upregulated in SCNT *B. taurus* placentas, with its promoter region exhibiting hypomethylation, increased H3K4me3 modification, and enhanced chromatin accessibility. Targeted knockdown of *Idh2* via CRISPR-Cas9 or inhibition of α -KG production using specific inhibitors effectively reversed these epigenetic abnormalities and improved the placental hypertrophy phenotype. In *Mus musculus* validation experiments, maternal α -KG supplementation during pregnancy induced placental hypertrophy, whereas inhibiting *Idh2* activity completely rescued this phenotype. This study is the first to elucidate the molecular mechanism by which *Idh2*- α -KG axis regulates placental development through a "metabolism-epigenetics" axis, providing novel strategies to improve cloning efficiency and potential intervention targets for treating placenta-related diseases (e.g., placental macrosomia).

Keywords: Somatic cell nuclear transfer; *Idh2*- α -KG axis; Placental hypertrophy; Metabolism-epigenetics regulation; DNA/histone methylation; Imprinted genes

INTRODUCTION

The placenta, as a crucial organ at the maternal–fetal interface during pregnancy, plays a vital role in the healthy growth of the fetus through its normal development (Burton

et al., 2016). Although the SCNT technique has been successfully used to clone a variety of mammals (Loi et al., 2016; Matoba et al., 2018b; Wilmut et al., 1997), abnormal phenotypes such as placental hypertrophy result in a pregnancy success rate of less than 2% for cloned mammals, especially ruminant mammals (Constant et al., 2006; Ogura et al., 2013; Silveira et al., 2019; Smith et al., 2012). This seriously limits the application value of this technology in agriculture and the conservation of endangered species. This phenomenon not only occurs in cloned animals but also shows similar pathological features in human pregnancy disorders such as pre-eclampsia (Burton et al., 2019). This indicates that abnormal conditions in cloned placentas may provide an important model for studying human pregnancy complications.

Current research has shown that although inhibiting histone deacetylation, reducing DNA methylation modifications, and eliminating persistent H3K9me3 modifications have to some extent improved the developmental competence of SCNT embryos, abnormal large placentas are still observed in the surviving embryos (Gao et al., 2018; Kishigami et al., 2006; Matoba et al., 2014). This indicates that the current epigenetic reprogramming strategies have not yet been able to completely overcome the obstacles to placental development in cloned animals. Certain progress has been made in the research of abnormal placental hyperplasia in cloned animals. Matoba (2019) et al. found that the dysregulation of epigenetic modification of the H3K27me3-dependent imprinted gene solute carrier family 38 member 4 (*Slc38a4*) excessively activated the mTORC1 signaling pathway, which in turn promoted amino acid transport and ultimately led to the hyperplasia of the placenta in SCNT *M. musculus*. Tanaka

(2001) et al., confirmed that the abnormal expansion of the placental spongiotrophoblast layer was the cause of the enlarged placenta in cloned *M. musculus*. Additionally, it was found that there were significant abnormalities in the levels of epigenetic modifications such as 5mc, 5hmc, H3K4me3, and H3K9me3 in cloned *B. taurus* placentas (Arnold et al., 2017; Silveira et al., 2019). During pregnancy, the placenta, as a highly active metabolic and endocrine organ, and the efficiency of nutritional distribution directly determines the outcome of fetal development (Yu et al., 2024). Studies have shown that the fetal placenta can take up 30% of glucose from the maternal circulation. Moreover, placental dysfunction induced by metabolic disorders is closely linked to pre-eclampsia, and fetal growth restriction (Aldoretta et al., 1999). Although the abnormal hyperplasia of SCNT placentas is related to epigenetic defects such as DNA methylation disorders (Liu et al., 2008; Matoba et al., 2014), abnormal histone modifications (Gao et al., 2018), and dysregulation of imprinted gene expression (Inoue et al., 2017; Xing et al., 2007), the upstream metabolic regulatory mechanisms driving these abnormalities remain unclear. In particular, the molecular pathways through which intermediate products during energy metabolism affect placental development via the “metabolism-epigenetic modification” axis remain unclear.

In recent years, significant breakthroughs have been made in the research of metabolic reprogramming, revealing the crucial regulatory role of metabolic intermediates in cell fate determination. Studies have found that the metabolic characteristics of embryonic stem cells are closely related to their pluripotent state. During the self-renewal stage, embryonic stem cells rely on glycolysis, while during

89 differentiation they switch to oxidative phosphorylation. The acetyl-CoA produced
90 during this metabolic transition can directly affect the state of epigenetic modification
91 by regulating the level of histone acetylation (Aplin et al., 2020). As a core metabolite
92 of the tricarboxylic acid cycle (TCA), α -KG has multi-dimensional regulatory functions.
93 A large number of studies have shown that α -KG can serve as a key cofactor for various
94 epigenetic modifications, including the activities of TET dioxygenases and KDMs, and
95 participate in cell fate determination by dynamically regulating the state of chromatin
96 modification (Carey et al., 2015; Huang et al., 2023; Rasmussen et al., 2016; TeSlaa et
97 al., 2016; Wu et al., 2017; Zhang et al., 2015). Specifically, on the one hand, in the
98 presence of Fe^{2+} and oxygen, the α -KG-dependent TET enzymes can catalyze the
99 conversion of 5mc to 5hmc, thereby promoting active DNA demethylation. This
100 process is crucial for maintaining the hypomethylated state of pluripotency genes (such
101 as *Nanog* and *Oct4*) in embryonic stem cells (ESCs) (Carey et al., 2015; Ito et al., 2011).
102 On the other hand, α -KG-dependent JMJD3 can remove the inhibitory histone mark
103 H3K27me3 and activate the expression of genes related to differentiation. The nuclear
104 α -KG concentration gradient is regulated by glucose metabolism, which further affects
105 the efficiency of JMJD1A in removing H3K9me2, thus forming a positive feedback
106 loop of nutrient sensing-epigenetics (Agger et al., 2007; Gao et al., 2023; Reid et al.,
107 2017). In addition, α -KG can stabilize HIF-1 α by inhibiting hypoxia-inducible factor
108 prolyl hydroxylase (PHD), coordinate hypoxic adaptation, and regulate histone
109 succinylation modification through its metabolic derivative succinyl-CoA (Koivunen
110 et al., 2007). Recent studies have also revealed that α -KG can coordinate cell

proliferation and senescence by activating the mTOR signaling pathway or inhibiting ATPase activity (Chin et al., 2014). Notably, in SCNT embryos, abnormal α -KG metabolism may lead to incomplete erasure of “epigenetic memory” (Xie et al., 2022). However, this hypothesis has not yet been verified in placental development. Currently, most of the research on cloned placentas focuses on imprinted genes (Inoue et al., 2017) and hormone secretion (Constant et al., 2006), while the understanding of the metabolic-epigenetic regulatory axis is relatively limited.

IDH2, a key rate-limiting enzyme in the TCA cycle, together with cytoplasm/peroxisome IDH1 and mitochondrial IDH3, constitutes a metabolic regulatory network. IDH2 catalyzes the reversible conversion of isocitrate to α -KG with nicotinamide adenine dinucleotide phosphate (NADP(H)) as a cofactor and plays a central role in maintaining the cellular redox balance (NADP⁺/NADPH) and metabolic reprogramming (Han et al., 2017; Jo et al., 2001). Current studies have shown that abnormal expression of Idh2 significantly alters the metabolic phenotype of lung cancer cells. Its overexpression activates the hypoxia-inducible factor 1 alpha (HIF1 α) signaling pathway, leading to enhanced glycolysis, accelerated glutaminolysis, and lactate accumulation, while simultaneously inhibiting adenosine triphosphate (ATP) synthesis (Li et al., 2018). Specific inhibition of Idh2 significantly reduces the flux of the reductive TCA cycle and affects physiological processes such as insulin secretion (Wang et al., 2023). Moreover, Idh2 hinders the formation of memory T cells in CAR-T cell therapy by restricting glucose utilization in the pentose phosphate pathway (PPP) and inhibiting histone acetylation. The Idh2 inhibitor Enasidenib can reverse this

process and enhance the anti-tumor immune efficacy (Si et al., 2024). In acute myeloid leukemia (AML), the Idh2^{R140Q} mutation leads to the accumulation of the oncogenic metabolite D-2-hydroxyglutarate (D2HG), thereby causing cell differentiation arrest. Idh2 inhibitors can effectively reduce the level of D2HG and restore the differentiation potential of cells (de Botton et al., 2023; Stein et al., 2017). These studies have revealed the dual regulatory role of Idh2 in metabolic diseases and tumorigenesis (Dang et al., 2016), but its role in placental metabolic reprogramming remains unclear.

Based on the above background, this study aims to systematically explore the regulatory mechanism of the Idh2- α -KG metabolic axis during the placental development of SCNT *B. taurus*, clarify the role of metabolic-epigenetic crosstalk in abnormal placental hypertrophy, explore potential intervention targets to improve cloning efficiency, and provide a theoretical reference for mechanistic studies of human placenta-related diseases.

MATERIALS AND METHODS

Ethics statement

All animals used in this study were handled in accordance with the guidelines of the China Welfare Committee. All experimental protocols were approved by the Animal Care and Use Committee of Inner Mongolia University (Approval No: IMU-CATTLE-2022-079).

Animals

The CON *B. taurus* and SCNT *B. taurus* selected in this study were housed at the base of Nei Da Sheng Mu High-Tech Animal Husbandry Company in Helinge'er District,

Hohhot ((E111°85', N40°55', 1040 m above sea level)). The donor cells selected for SCNT *B. taurus* were ear tip fibroblasts from a 2-year-old high-quality bull. All SCNT embryos were derived from the same bull and the same cell line to minimize individual and cellular background variations. The cells used for nuclear transfer were at the 3rd passage, displaying stable status and favorable proliferative activity. The CON *B. taurus* were pregnant cows artificially inseminated at the same time. The *B. taurus* herd was raised under the standard feed and breeding conditions on the ranch (Schuettengruber et al., 2017). The selection and production methods for CON and SCNT *B. taurus* are described elsewhere (Wu et al., 2025). Three CON *B. taurus* and three SCNT *B. taurus* at the same gestational stage were selected respectively. When the *B. taurus* gave birth, the placentomes of CON calves and SCNT calves born during the same gestational period were collected. First, the removed placentomes were rinsed with normal saline and divided into three aliquots. One aliquot was fixed in 4% paraformaldehyde for histological sectioning. A second aliquot was placed in phosphate-buffered saline (PBS, P4474, Sigma, Germany) containing 2% penicillin-streptomycin (PS, 15140122, Gibco, USA) for primary culture of placental mesenchymal cells. The remaining aliquot was snap-frozen in liquid nitrogen and stored at -80°C for subsequent molecular experiments.

Hematoxylin and eosin (HE) staining and morphological analysis of tissue sections

After the placentomes of CON and SCNT *B. taurus* and the *M. musculus* placental tissues under different treatments were fixed in 4% paraformaldehyde for 24h, tissue sections with a thickness of 5 µm were prepared. After dewaxing, hematoxylin-eosin

staining was used for histological morphological analysis. For the HE sections of CON and SCNT *B. taurus* placentas, an optical microscope (model, manufacturer) was used to randomly select 10 non-overlapping fields of view (0.3 mm² per field of view) on each section at a magnification of 200× for image acquisition. ImageJ software (v.1.53, NIH) was used to quantitatively analyze the number and morphological characteristics of placental trophoblast cells. SlideViewer software was used for image acquisition of mouse placental tissues.

RNA-seq sequencing and analysis

According to the manufacturer's procedure, total RNA was extracted from the placentomes of CON and SCNT *B. taurus* using Trizol reagent (16096020, Invitrogen, USA). The concentration and purity of total RNA were detected using Nanodrop 2000 (Thermo, USA), and the integrity of RNA was detected by agarose gel electrophoresis. cDNA libraries were generated following the mRNA-seq transcriptome library preparation protocol Illumina® Stranded Mra Prep, Ligation (Illumina, USA). There were 3 biological replicates in each group, and a total of 6 libraries were constructed. Then the synthesized cDNA was subjected to end-repair, phosphorylation and 'A' base addition according to Illumina's library construction protocol. Libraries were size selected for cDNA target fragments of 300 bp on 2% Low Range Ultra Agarose followed by PCR amplified using Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantified by Qubit 4.0, paired-end RNA-seq sequencing library was sequenced with the NovaSeq 6000 sequencer (2×150bp read length). The reads were mapped to the bovine reference genome (ARS-UCD1.2) using the alignment software Bowtie. To

identify differential expression genes (DEGs) between two different samples, the expression level of each transcript was calculated according to the transcripts per million reads (TPM) method. Differential expression analysis was performed using the DEG-Seq software. DEGs with $P\text{-Value} < 0.05$ and $|\text{Log}_2 \text{ fold change}| \geq 1$ were considered to be significantly different expressed genes. Gene ontology (GO) functional enrichment and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses of DEGs were performed using Goatools (v.1.2.3) and Python scipy (v.1.9.1) software, respectively.

Metabolomics sequencing and analysis

Total metabolites were extracted from the placentomes of CON and SCNT *B. taurus*. There were 3 biological replicates in each group, and a total of 6 libraries were constructed. For each gram of tissue sample, 3 mL of methanol and 0.64 mL of water were added and mixed well on ice. The samples were centrifuged at $15\,000 \times g$ for 10 min at 4°C , and the lower organic layer was collected for LC-MS analysis (LC-Bio, China). The metabolites were separated using a chromatographic column. The separated metabolites were analyzed by a mass spectrometer in both positive and negative ion modes. The obtained raw data were pre-processed through procedures such as filtering, value imputation, normalization, and logarithmic transformation. Metabolites were annotated using KEGG and human metabolome database (HMDB). Differential metabolites were screened based on a combination of univariate statistical analysis (t -test), multivariate statistical analysis (OPLS-DA/PLS-DA), and fold-change values (FC). The default screening criteria were $P < 0.05$, $\text{VIP} > 1$, and $(\text{FC} < 1 \text{ or } \text{FC} > 1)$.

Pathway enrichment analysis of the screened differentially expressed metabolites was performed using the KEGG (<http://www.genome.jp/kegg/>).

***Bos taurus* placental mesenchymal cell culture**

The primary culture of placental mesenchymal cells from CON and SCNT *B. taurus* was carried out following a previously established method (Wu et al., 2025). Briefly, fetal cotyledon-derived chorionic tissues from bovine placentomes were collected under aseptic conditions. After being thoroughly rinsed with PBS containing 2% PS, the tissues were mechanically minced into 1-3 mm³ tissue pieces. A combined enzyme digestion method was employed (a 1:1 volume ratio mixture of 0.25% trypsin, 59429C, Gibco, USA, and 1% collagenase type IV, 17104019, Gibco, USA). The digestion was performed three times at 37°C (30min each time), with intermittent shaking to promote dissociation. After the digestion was terminated, the cell suspension was collected by filtering through a 200-mesh cell sieve and centrifuged at 1500 r/min for 5min to obtain the cell pellet. For primary culture, DMEM/F12 medium (A4192001, Gibco, USA) supplemented with 20% fetal bovine serum (FBS, 10099141, Gibco, USA) and 1% PS was used. The cells were cultured at 37°C in an atmosphere of 5% CO₂ with saturated humidity. When the confluence of adherent cells reached 90%, the cells were digested for sub-culturing. Cells at passages 3-5 were used in the experiments. For drug intervention experiments, the concentrations of 10 µmol/L Enasidenib mesylated (S4929, Selleck, USA, purity: 99.42%) and 4 mmol/L dm-α-KG (349631, Sigma, Germany, purity: 96%) were selected based on preliminary dose-response assays, which showed optimal effects on regulating α-KG levels and epigenetic modifications

243 without affecting cell viability.

244 **Detection of the contents of *B. taurus* metabolites α -KG and SAM**

245 A commercial ELISA kit (for *B. taurus* α -KG, MM-5107801, for *B. taurus* SAM, MM-
246 5108101, Enzyme Immunoassay Biology, China) was used to quantitatively detect the
247 contents of α -KG and SAM in *B. taurus* placentomes and mesenchymal cells. The
248 specific steps are as follows: Sample pre-treatment: After homogenizing the tissues,
249 centrifuge at $12\,000\times g$ for 15min at 4°C and collect the supernatant. Centrifuge the cell
250 lysate directly. Detection procedure: After equilibrating the reagents at room
251 temperature for 20min, add 50 μL of standard substances with gradient concentrations
252 to the standard wells, add 10 μL of test sample mixed with 40 μL of diluent into sample
253 wells, and add only diluent into blank wells. Reaction system: Add 100 μL of HRP-
254 labeled detection antibody to each well and incubate at 37°C for 60min. Washing: Wash
255 the plate 5 times with $1\times$ washing solution. Color development: Add TMB substrate (50
256 μL /well) and react at 37°C in the dark for 15min, then add the stop solution to terminate
257 the reaction. Reading: Use a Varioskan™ LUX multimode microplate reader
258 (VL0000D0, Thermo, USA) to measure the absorbance (OD value) at a wavelength of
259 450 nm, and calculate the concentration according to the standard curve. Set 3 technical
260 replicates for each sample, and present the data as mean $\pm$ SEM.

261 **Detection of α -KG content in *M. musculus***

262 In this study, an α -KG ELISA kit (SPS-20562, Saipaisen Biology, China) was used to
263 measure the α -KG content in *M. musculus* serum and tissues. The specific operation
264 steps and evaluation methods were all carried out according to the kit instructions. The

brief procedure is as follows: Collect *M. musculus* blood and centrifuge it at 1500×g for 15min to separate the serum. Collect placental tissues from mice under different treatments, grind them into tissue homogenates, centrifuge at 3000 r/min for 10min, and collect the supernatants. Then perform the reaction steps as required by the instructions. Use standard substances with a concentration gradient of 0, 4, 8, 16, 32, and 64 ng/mL to draw a standard curve. Detect the OD value at 450 nm using a microplate reader, and calculate the final α-KG content according to the linear regression formula of the standard curve.

Cell immunofluorescence

After the placental mesenchymal cells of CON *B. taurus* and SCNT *B. taurus* were cultured to 90% confluence, they were fixed with 4% paraformaldehyde (P0099, Beyotime, China) at room temperature for 10min. After permeabilization treatment with 0.1% Triton X-100 (X100, Sigma–Aldrich, Germany) (3×10min), they were blocked with 1% BS-PBS for 1h. First, the samples were incubated with the primary antibody (Supplementary Table S1) at 4°C overnight, and then incubated with the secondary antibody (Supplementary Table S1) at room temperature for 1h. Nuclear staining was performed by incubating with DAPI (1 μg/mL, D9542, Sigma-Aldrich, Germany) for 15min, and the mounting medium was ProLong Gold antifade reagent (P36934, Thermo, USA). Images of all samples were acquired using a Nikon A1 plus confocal microscope (Japan) under the same parameters. At least 10 random fields of view were analyzed for each group, and image processing was performed using NIS-Elements AR software (v.5.11).

Western blot analysis

Total protein was extracted from placentomes of CON and SCNT *B. taurus*, cultured placental mesenchymal cells under different treatments, and *M. musculus* fetal tissues using RIPA lysis buffer (P0013C, Beyotime, China). Protein concentration was determined using a BCA protein assay kit (23227, Thermo, USA). An appropriate volume of protein supernatant was mixed with 5×loading buffer (P0015, Beyotime, China) containing DTT at a volume ratio of 4:1, followed by denaturation in a boiling water bath for 10min. SDS-PAGE was performed according to the instructions of the gel preparation kit, with an appropriate separating gel concentration selected based on the molecular weight of the target protein. After separation by SDS-PAGE, proteins were transferred to a PVDF membrane (23227, Millipore, Germany). The membrane was blocked with 5% non-fat milk at room temperature for 1h. The blocked membrane was then incubated with primary antibodies (Supplementary Table S1) overnight at 4°C. Following primary antibody incubation, the membrane was washed three times with 1×TBST washing buffer on a shaker for 5min each. Subsequently, the membrane was incubated with the corresponding secondary antibodies (Supplementary Table S1) with gentle shaking at room temperature for 1h. The washed membrane was placed in a self-sealing bag, and a 1:1 mixture of Luminol Reagent and Peroxide Solution chemiluminescent developer was added dropwise. After removing air bubbles, the membrane was exposed and imaged using a chemiluminescence imaging system. Gray values were analyzed using ImageJ software (National Institutes of Health, USA) to quantify protein expression levels.

Dot blot

The TIANamp Genomic DNA Kit (W9104, TIANGEN, China) was used to extract genomic DNA from the placentomes of CON and SCNT *B. taurus*, mesenchymal cells, and placental tissues of *M. musculus* under various treatments. The quality of the DNA was detected using a Nanodrop micro-spectrophotometer (ND-ONE-W, Thermo Scientific, USA). After the DNA samples were diluted to different concentrations with TE buffer, they were heat-denatured at 100°C for 10min and immediately cooled on ice. Then, 2 µL of denatured DNA at different concentrations was spotted onto a nylon membrane (YA1760, Solarbio, China) and fixed by UV cross-linking for 5min. The membrane was blocked by incubating with 5% non-fat milk (w/v, dissolved in TBST) on a shaker at room temperature for 1h, and then incubated with the primary antibody (Supplementary Table S1) at 4°C overnight. The steps of secondary antibody incubation and chemiluminescence detection refer to the standard Western blot procedure.

Use the CRISPR-Cas9 technology to construct an Idh2 knockdown vector

The Idh2 gene is located on *B. taurus* chromosome 11 and contains 11 exons. We used SnapGene software to design sgRNAs targeting the first four exons, and the primer sequences are shown in the supplementary table S2. The pSpCas9BB-2A-GFP (PX458) (48138, Addgene, USA) vector was digested with Bbs I enzyme, and the sgRNA was ligated to the vector using Solution I. The ligated product was transformed into Stbl3 Escherichia coli competent cells. After bacterial preservation and sequencing, the Idh2 knockdown vector can be obtained.

Cell transfection

Transfection was performed using Lipofectamine 2000 (11668019, Invitrogen, USA) according to the manufacturer's instructions. The successfully constructed sh-Idh2 vector was transfected into placental mesenchymal cells of CON and SCNT *B. taurus*. Six hours after transfection, the medium was replaced with complete medium, and the cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂ for 48h. Then, the fluorescence expression was observed using an inverted fluorescence microscope (ZEISS, Germany). The expression level of Idh2 in placental mesenchymal cells after transfection with the sh-Idh2 vector was detected by RT-qPCR and Western blot.

CCK8 cell proliferation assay

Placental mesenchymal cells were seeded into a 96-well plate at a density of 2×10^3 cells per well in 100 µL, and then cultured at 37°C in an atmosphere containing 5% CO₂ for 24h, 48h, 72h, 96h, and 120h respectively. According to different culture times, 10 µL of CCK8 (C0037, Beyotime, China) reagent was added to each well, followed by incubation at 37°C in an atmosphere containing 5% CO₂ in the dark for 2h. The optical density (OD) value at 450 nm was measured using a microplate reader.

RT-qPCR gene expression detection

Total RNA was extracted from the placentomes of CON *B. taurus* and SCNT *B. taurus*, *B. taurus* placental mesenchymal cells under different treatments, and *M. musculus* placental tissues using TRIzol reagent (16096020, Invitrogen, USA) according to the manufacturer's instructions. The extracted RNA was reverse-transcribed into Cdna

using HiScript® II Q RT SuperMix for Qpcr (+Gdna wiper) (R223-01, Vazyme, China). Detection was performed using a Light Cycler 480 II real-time fluorescent quantitative PCR instrument (Germany) in combination with ChamQ Universal SYBR Qpcr Master Mix (Q711-02, Vazyme, China). The data were analyzed using the $2^{-\Delta\Delta CT}$ method and normalized with Gapdh as an internal reference. The primer information is detailed in the supplementary table S3.

WGBS library construction and sequencing

The genomic DNA of placentomes from CON and SCNT *B. taurus* and treated placental mesenchymal cells was extracted using the TIANamp Genomic DNA Kit (W9104, TIANGEN, China). The extracted DNA was detected by 1% agarose gel electrophoresis and Qubit 4.0 fluorescence quantification (DNA integrity index > 7, total DNA extraction amount $\geq 15 \mu\text{g}$).

For library construction, referring to the Illumina TruSeq DNA Methylation standard protocol, the DNA was fragmented to 350 bp using an ultrasonic crusher. The KAPA HyperPrep Kit was used for end-repair, addition of base A and sequencing adapters. Subsequently, the EZ DNA Methylation-Gold Kit (Zymo Research, USA) was used for bisulfite conversion to convert unmethylated C in the DNA fragments to U, with a conversion efficiency of > 99%. Notably, conventional WGBS cannot distinguish 5mC from 5hmC, and the detected methylation signals represent the combined levels of these two modifications. After 12 cycles of PCR amplification, fragments of 300-400 bp were selected using AMPure XP beads. A total of 6 libraries were constructed and sequenced on the Illumina HiSeq X Ten platform with 150-bp

paired-end reads. After quality control of the raw data by FastQC, low-quality bases and adapter sequences were removed using TrimGalore (v.0.6.6). The reads were aligned to the bovine reference genome ARS-UCD1.2 using Bismark (v.0.22.3).

The analysis of methylation levels was as follows: The methylation rates of CG, CHG, and CHH sites were calculated. The DSS software (v.2.42.0) was used to identify differentially methylated regions (DMRs) (screening criteria: $P < 0.00005$, methylation difference $\geq 10\%$, containing ≥ 3 CpG sites). The KEGG database was used for signal pathway enrichment analysis of the hypermethylated (Hyper) and relatively hypomethylated (Hypo) DMR regions. The Integrative Genomics Viewer (IGV, <http://software.broadinstitute.org/software/igv/>) was used to analyze the distribution of sequencing reads across the whole genome and specific genes.

Chromatin immunoprecipitation (ChIP)-seq library construction and sequencing

ChIP assays were performed using the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) (9005, Cell Signaling Technology, USA). The specific procedure was as follows: Cells were cross-linked with 1% formaldehyde for 10min at room temperature, and then the reaction was terminated with 125 mmol/L glycine. Micrococcal Nuclease was used to digest the chromatin at 37°C for 15min to obtain chromatin fragments of 200-400 bp. For each reaction, 5 µg of chromatin was incubated with 2 µg of specific antibodies (Supplementary Table S1) overnight at 4°C, and IgG was used as a negative control.

The NEBNext® Ultra™ II DNA Library Prep Kit (NEB) was used for end-repair, A-tailing, adapter ligation, and 12 cycles of PCR amplification. A total of 12

libraries (3 modifications×4 groups) were constructed. After passing the quality control by Qubit and Agilent 2100, these libraries were sequenced on the Illumina NovaSeq 6000 platform with 150-bp paired-end reads. The raw data were subjected to quality filtering using Fastp (v.0.23.2) to remove low-quality bases and adapter sequences. Bowtie (v.2.4.4) was used to align the reads to the reference genome (ARS-UCD1.2). Peak calling was performed using MACS2 (v.2.2.7.1) with a Q -value<0.05, and deep Tools (v.3.5.1) was used to generate the whole-genome signal distribution map. The IGV (<http://software.broadinstitute.org/software/igv/>) was used to analyze the distribution of sequencing reads across the whole genome and specific genes.

Assay for transposase-accessible chromatin (ATAC) sequencing

Nucleus preparation: CON and SCNT *B. taurus* placentomes were collected, with three replicates set for each group. The placentomes were digested into single-cell suspensions using collagenase, and the cell viability was detected to obtain cell suspensions whose cell viability met the sequencing criteria. The cells were washed three times with PBS. The cell pellet was placed on a rotating mixer and lysed at 4°C for 10min. The Tn5-transposed DNA was purified using AMPure DNA magnetic beads. The appropriate number of PCR cycles was determined. An amplification program was run using the Agilent TapeStation 2200 instrument to construct libraries. A total of 6 libraries were constructed.

Data analysis: The raw sequencing sequences were filtered using the Trimmomatic (v.0.36) software to obtain high-quality Clean Reads. The filtered sequences were aligned to the reference genome using Bowtie2 (v.2.2.6), and the reads aligned to the

organelle genomes were filtered out using remove Chrom. PCR duplicates were removed using Picard (v.1.126), and the number of mapped reads was down sampled to a standard of approximately 20 M. Peak calling was performed using the MACS2 software. IDR evaluation was carried out on the peaks between each sample, with the IDR threshold set at 0.05. Differential accessible regions (DARs) were obtained from the commonly identified peak set using DESeq2 (v.1.28.1), with the screening criteria of $|\log_2 \text{fold change}| < 0.5$ and nominal P -value > 0.05 . IGV was used to analyze the ATAC sequencing tracks.

***Mus musculus* α -KG feeding experiment**

In this study, 7-8-week-old female Kunming white *M. musculus* (weighing 20 ± 2 g) were selected. These *M. musculus* were purchased from Cyagen and acclimated for 1 week in a specific pathogen-free (SPF) barrier environment (temperature, $22 \pm 1^\circ\text{C}$, humidity, $50 \pm 5\%$, 12h light/dark cycle). The female *M. musculus* were randomly divided into 4 groups, with 10 mice in each group. Superovulation was induced by subcutaneous injection of pregnant mare serum gonadotropin (PMSG, G4877, Sigma-Aldrich, Germany, 10 IU per mouse) and subsequent intraperitoneal injection of human chorionic gonadotropin (hCG, C8554, Sigma-Aldrich, Germany, 10 IU per mouse) 47.5h later. Then, the female *M. musculus* were caged with male Kunming white *M. musculus* at a ratio of 1:1. The formation of vaginal plugs was checked 12-13h later. After the detection of vaginal plugs (on embryonic day 0.5, E0.5), the experimental groups were set as follows, Control group (provided with regular drinking water); dm- α -KG continuous group, provided with a 0.75% α -KG (349631, Sigma-Aldrich,

441 Germany aqueous solution from E0.5 to E19; α -KG withdrawal group (treated with α -
442 KG from E0.5 to E10 and then switched to regular water); α -KG inhibition group
443 (treated with α -KG from E0.5 to E10 and then supplemented with 10 mg/kg/d
444 Enasidenib mesylate (HY-18769, MedChemExpress, USA). On E16 and E19 of
445 pregnancy, the *M. musculus* were euthanized by cervical dislocation. After completely
446 separating the placental and fetal tissues, the weights were measured using a Sartorius
447 electronic balance (v. CPA225D, accuracy 0.01 mg). The morphological characteristics
448 of the mouse fetuses and placentas were observed, photographed, and recorded. The
449 tissue samples were stored in liquid nitrogen or 4% paraformaldehyde (G1101,
450 Servicebio, China) for subsequent relevant experimental verification.

451 Construction and detection of *Idh2*^{+/-} *M. musculus*

452 In this study, 8-week-old C57 BL/6 wild-type (WT) and *Idh2* gene-knockout
453 heterozygous (*Idh2*^{+/-}) *M. musculus* (purchased from Cyagen, agreement number,
454 KOAIP240201WX1) were selected. WT×WT and *Idh2*^{+/-}×*Idh2*^{+/-} matings were carried
455 out respectively. Genotyping was performed using two pairs of specific primers: Primer
456 set 1 (F: 5'-ACTGGAATAAGTGGGTTTGCCTG-3'; R: 5'-
457 GCTCTGTCCAGGTTGCTCTTAATG-3'), which amplified a 538-bp fragment
458 representing the mutant allele; Primer set 2 (F: 5'-
459 GTACCAACCCTATTGCCAGCATC-3'; R: 5'-
460 GCTCTGTCCAGGTTGCTCTTAATG-3'), which amplified a 752-bp fragment
461 representing the wild-type allele. The pregnant *Idh2*^{+/-} *M. musculus* were randomly
462 divided into two groups, with 10 *M. musculus* in each group. The experimental group

was provided with an aqueous solution containing 0.75% dm- α -KG for drinking during pregnancy (starting from E0.5), while the control group was provided with ordinary water. The body weights of the mother *M. musculus* were recorded every 48h during pregnancy. At E16.5, after anesthesia, blood was collected by cardiac puncture. After serum separation, the *M. musculus* were euthanized. The placental and fetal tissues were completely removed, weighed, and photographed. Subsequently, the placental tissues were divided into three parts, one part was fixed in 4% paraformaldehyde for HE staining to observe morphological changes, another part was snap-frozen in liquid nitrogen for RT-qPCR to detect gene expression; the third part was lysed with RIPA to extract total protein, and DNA was also extracted respectively. The modification levels of H3K4me3/H3K9me3/H3K27me3 were detected by Western blot, and the methylation status of 5mC/5hmC was analyzed by Dot blot. The α -KG content in the serum and placental tissues was detected by ELISA.

Statistical analysis

All data were expressed as mean $\pm$ SEM. GraphPad Prism 8 software was used to analyze the significance of difference (one-way ANOVA was used for multiple group comparisons and Student's *t*-test was used for two-group comparisons) and $P < 0.05$ was considered a significant difference or as indicated in the figure legends. The experiment was repeated three times for both technical and biological replicates. Fetal and placental weights were analyzed as the mean value per litter, and data are presented as mean $\pm$ SEM of all fetuses or placentas per litter.

RESULTS

Abnormal epigenetic modifications were found in the placentas of SCNT *B. taurus*

In our previous study, we observed that SCNT *B. taurus* during pregnancy exhibited significant characteristics of placental hypertrophy, including an increase in abdominal circumference and an enlargement of placental cotyledons. Additionally, we pointed out that this abnormality was closely related to the dysregulation of placental cell proliferation and cell cycle caused by YAP1 through the regulation of CCND1-CDK6 expression (Wu et al., 2025). In this study, through the analysis of HE-stained sections, it was found that the placentas of SCNT *B. taurus* had histological abnormalities, such as the loosening of interdigitation between the chorion villi and uterine caruncle epithelial cells, the increase in the number of trophoblast binucleate cells and crypt giant cells, the expansion of crypt area, and the hyperplasia of placental mesenchyme (Supplementary Figure S1A; Supplementary Table S4). Given that previous studies have confirmed that abnormal DNA and histone methylation modifications are one of the key factors leading to placental hypertrophy in SCNT *M. musculus* (Gao et al., 2018; Wang et al., 2020a), we systematically compared the epigenetic characteristics of placentomes and mesenchymal cells between CON and SCNT *B. taurus*. The results showed that the modification levels of H3K4me3, 5mc, and 5hmc were significantly increased, while the modification levels of H3K9me3 and H3K27me3 were decreased, with the change in 5hmc being particularly significant (Figure 1A–I). These epigenetic changes were accompanied by a significant enhancement in the proliferation of placental mesenchymal cells in SCNT *B. taurus* (Figure 1J). To explore the molecular mechanism of this phenomenon, we used RNA-seq technology for analysis (Figure 1K).

507 Among the 12 145 co-expressed genes, 3 368 differentially expressed genes were
508 identified (including 1 787 down-regulated genes and 1 581 up-regulated genes)
509 (Supplementary Figure S1B, C). The heatmap analysis showed that the expression
510 levels of enzymes related to DNA methylation (DNMT3B, TET3) and histone
511 demethylases (KDM4A/B, KDM5B/C, KDM6A/B) in SCNT placentomes were
512 significantly higher than those in the control group (Figure 1L). This result was
513 confirmed at both the mRNA and protein levels (Figure 1D–H; 1M–Q), providing
514 crucial clues for elucidating the epigenetic regulatory mechanism underlying abnormal
515 placental development in SCNT *B. taurus*.

516 **The increase in α -KG mediated by Idh2 affects epigenetic modification in the**
517 **placenta of SCNT *B. taurus***

518 Through KEGG pathway enrichment analysis of differentially expressed methylated
519 genes in the placentas of CON and SCNT *B. taurus*, it was found that these genes were
520 mainly enriched in pathways such as cysteine and methionine metabolism, retinol
521 metabolism, and steroid hormone biosynthesis (Figure 2A). Given that energy
522 metabolism has been confirmed directly or indirectly regulate epigenetic modifications
523 in embryos and stem cells (Ispada et al., 2020), and considering the abnormal DNA and
524 histone methylation observed in cloned placentas, we further explored the potential
525 association between them and energy metabolism. Metabolomic analysis of
526 placentomes was performed using LC-MS technology (Figure 1K; Supplementary
527 Figure S2A). A total of 483 and 539 differential metabolites were identified in positive
528 and negative ion modes, respectively (Figure 2B; Supplementary Figure S2B).

Heatmap clustering analysis showed that the glutamate level in placentomes of the SCNT group was significantly increased compared to the CON group, while the succinate level was decreased (Figure 2C; Supplementary Figure S2C). Notably, as the precursor and product of the synthesis of α -KG, a key metabolite in the TCA cycle, respectively, changes in the content of these two metabolites may lead to the accumulation of α -KG (Figure 2D). Subsequent tests confirmed that the content of α -KG in both the placentomes and mesenchymal cells of SCNT *B. taurus* was significantly higher than that of CON *B. taurus* (Figure 2E, F). In addition to participating in energy metabolism, α -KG can also serve as a key molecule for epigenetic regulation. It affects the DNA and histone demethylation processes by regulating the activities of TETs and KDMs dioxygenases. Meanwhile, the methyl donor SAM regulates the activity of DNMTs to influence methylation modifications (Tischler et al., 2019). Although the content of SAM in the placentas of SCNT *B. taurus* showed an upward trend, this difference did not reach a statistical significance (Supplementary Figure S2D, E). This might be the reason for the relatively high level of 5mc in the placentas of SCNT *B. taurus*. To investigate the reason for the increase in α -KG levels in the placentas of SCNT *B. taurus*, we examined the expression of its key metabolic enzymes. The results showed that the expression of *Idh2* in the placentas of SCNT *B. taurus* was significantly up-regulated, indicating that the increase in α -KG levels in the placentas of SCNT *B. taurus* might be related to the up-regulated expression of *Idh2* (Figure 2G; Supplementary Figure S2F).

The naïve *Idh2*- α -KG metabolic axis regulate the proliferation and epigenetic

modification of placental mesenchymal cells in SCNT *B. taurus*

To elucidate the regulatory role of α -KG in the abnormal epigenetic modifications of SCNT *B. taurus* placentas, we conducted a drug intervention experiment during the culture of SCNT *B. taurus* placental mesenchymal cells. Firstly, 10 $\mu\text{mol/L}$ of the *Idh2*-specific inhibitor Enasidenib mesylate was added to the culture medium of the placental mesenchymal cells cultured for 3 days, and the treatment was continued for 72h. Subsequently, the medium was replaced with fresh culture medium containing 4 mmol/L cell membrane-permeable dm- α -KG, and the cells were further cultured for 48h. The cell samples were collected for subsequent analysis (Figure 3A). The results showed that treatment with 10 $\mu\text{mol/L}$ Enasidenib mesylate could significantly inhibit the expression of the *Idh2* gene. In subsequent experiments, the 10 $\mu\text{mol/L}$ inhibitor was added whole culture period (Figure 3B), which led to a decrease in the intracellular α -KG content to level comparable to that in placental mesenchymal cells of CON cattle (Figure 3C). In addition, the supplementation of 4 mmol/L dm- α -KG effectively restored the intracellular α -KG content (Figure 3D). This phenomenon was also verified in the placental mesenchymal cells of CON *B. taurus* (Supplementary Figure S3A). This is consistent with the previous study that dm- α -KG preferentially increased the intracellular α -KG level (Kang et al., 2020). Meanwhile, we found that the changes in α -KG levels significantly affected the expression patterns of various epigenetic markers. When the α -KG level decreased in the placental mesenchymal cells of SCNT *B. taurus*, the level of 5hmc modification significantly decreased to that of the CON group. At the same time, characteristic changes in histone modifications occurred, with the level of

H3K4me3 decreasing and the levels of H3K9me3 and H3K27me3 increasing. These epigenetic modification changes were accompanied by a significant reduction in cell proliferation ability (Figure 3E–N). After restoring the α -KG level by supplementing dm- α -KG, the abnormal epigenetic modifications were significantly improved, and the cell proliferation ability was also enhanced (Figure 3E–N). It is notable that, after treatment with dm- α -KG, the cells in the CON group showed a similar trend of changes in epigenetic modifications. This included increased modification levels of 5hmc and H3K4me3, decreased modification levels of H3K9me3 and H3K27me3, and enhanced cell proliferation ability (Supplementary Figure S3B–K). Further studies have shown that changes in α -KG levels could dynamically regulate the expressions of key epigenetic regulatory factors. In the SCNT group, the decrease in α -KG level led to significant down-regulation of the expression of epigenetic regulatory enzymes such as TET2, TET3, DNMT3B and KDM6B. After restoring the α -KG level, the expression of these genes significantly restored (Figure 3O–R). Similarly, in the CON group, the expressions of DNMT3B, TET3, and KDM6B in the cells were also significantly upregulated after dm- α -KG treatment (Supplementary Figure S3I–V). Collectively, these results confirm that α -KG plays a core regulatory role in regulating the epigenetic reprogramming of SCNT *B. taurus* placentas by epigenetic modification enzymes.

***Idh2* knockdown leads to alterations in α -KG-dependent epigenetic modifications in placental mesenchymal cells**

To investigate whether the changes in α -KG levels can affect the methylation modification of SCNT *B. taurus* placentas by regulating the expression of *Idh2*, we

employed the CRISPR-Cas9 gene editing technology, designed sgRNAs targeting the first four exons of the *Idh2* gene, and constructed and edited the vectors (Supplementary Figure S4A–C). By screening multiple sgRNAs, it was found that sgRNA#3 had the highest knockdown efficiency (Supplementary Figure S4D–F). The results showed that the knockdown of *Idh2* significantly reduced the intracellular α -KG level (Supplementary Figure S4G), and led to a marked decrease in cell proliferation ability (Supplementary Figure S4H). The detection of epigenetic modification levels revealed that the knockout of *Idh2* caused significant changes in DNA and histone methylation modifications. Specifically, the level of 5hmc modification decreased, while the level of 5mc modification increased. At the same time, histone modifications exhibited characteristic changes, with a decrease in H3K4me3 level, and an increase in H3K9me3 and H3K27me3 levels (Supplementary Figure S4I–K). From the perspective of molecular mechanism, *Idh2* gene knockout not only reduced the level of α -KG, but also significantly downregulated the expression levels of key epigenetic regulatory factors TET3, DNMT3B and KDM6B (Supplementary Figure S4L–N). Collectively, these results confirm that *Idh2* regulates the expression of enzymes related to epigenetic modifications by modulating the α -KG level, thereby changing the methylation modification status of cells and ultimately having a significant impact on cell proliferation ability.

α -KG regulates the expression of imprinted genes in the placenta of SCNT *B. taurus* through epigenetic reprogramming

We further elucidate the regulatory mechanism of α -KG in the placental hypertrophy

617 of SCNT *B. taurus* through multi-omics integrated analysis (Figure 4A). WGBS
 618 detection revealed that the DNA methylation levels in the placentomes and
 619 mesenchymal cells of SCNT *B. taurus* were significantly higher than those of CON *B.*
 620 *taurus*. Among them, the proportion of CG-type methylation reached 60.85% (47.43%
 621 in CON *B. taurus*) (Figure 4B–D; Supplementary Figure S5A–C). Analysis of
 622 differentially methylated regions showed that these regions were significantly enriched
 623 in Metabolic pathways (Supplementary Figure S5D, E). Meanwhile, treatment with the
 624 Idh2 inhibitor Enasidenib mesylate could reduce the DNA methylation level in the
 625 placental mesenchymal cells of SCNT *B. taurus* (mainly affecting CG-type
 626 methylation), and supplementation of α -KG could reverse this effect (Figure 4B–E).
 627 ChIP-seq analysis further revealed that the modification of H3K4me3 increased in the
 628 placenta of SCNT *B. taurus*, while the modifications of H3K9me3/H3K27me3
 629 decreased. Treatment with Enasidenib mesylate could reverse these histone
 630 modification changes, and supplementing α -KG could rescue these alterations (Figure
 631 4F–L). ATAC-seq analysis showed significant chromatin accessibility differences
 632 between the placentas of SCNT *B. taurus* and CON *B. taurus* (Supplementary Figure
 633 S5F, G). The accessibility of the whole-genome TSS regions in the placentas of SCNT
 634 *B. taurus* was significantly enhanced (Figure 4M; Supplementary Figure S5H), as
 635 shown on chromosome 1 (Figure 4N). Analysis of DARs identified 10 958
 636 downregulated regions and 1 301 upregulated regions, mainly enriched in the promoter
 637 regions (Figure 4O, P; Supplementary Figure S5I). Additionally, through multi-omics
 638 joint analysis, we confirmed the abnormal epigenetic modification level in the promoter

region of the *Idh2* gene and the high accessibility of the TSS region in the placentas of SCNT *B. taurus*. Specifically, the promoter region of the *Idh2* gene showed low levels of DNA methylation modification, low levels of H3K9me3/H3K27me3 modifications, and high levels of H3K4me3 modification. This unique epigenetic modification pattern is the reason for the high expression of *Idh2*, and this epigenetic modification is regulated by the α -KG content (Supplementary Figure S6).

Imprinted genes play an important role in the processes of gamete production, embryonic and placental growth and development in mammals (Scagliotti et al., 2021). Dysregulation of imprinted gene expression can lead to abnormalities in the proliferation, differentiation, and apoptosis of trophoblast cells, thereby causing placental growth defects (Fowden et al., 2011). Among the 29 bovine imprinted genes that have been confirmed in *B. taurus* to date, we found that 23 of them were differentially expressed in the placentomes of SCNT *B. taurus* (Figure 5A). The paternal and maternal imprinted genes exhibited abnormal expression patterns in the placentas of SCNT *B. taurus* (Figure 5B). Subsequently, we detected and analyzed the expression levels of imprinted genes that have been confirmed to play a crucial role in bovine placental development. The results showed that *Znf597* and *Igf2r* were highly expressed in the placentomes and mesenchymal cells of SCNT *B. taurus*, while *Phlda2*, *Peg10*, and *Magel2* were lowly expressed in the placentomes and mesenchymal cells of SCNT *B. taurus* (Figure 5C). However, the expression of *Snrpn* and *Magel2* showed no significant difference (Figure 5C). Treatment with Enasidenib mesylate can correct these abnormal expression patterns, and supplementation of α -KG can reverse this

661 corrective effect (Figure 5D). Through multi-omics integrated analysis, we found that
662 the dysregulation of imprinted gene expression in SCNT placentas is closely related to
663 multi-level epigenetic abnormalities. ATAC-seq analysis showed that the accessibility
664 of the promoter regions of *Phlda2*, *Igf2r*, *Peg10*, and *Znf597* changed significantly,
665 providing a structural basis for the abnormal gene transcription. Further analysis
666 revealed that these accessibility changes was related to abnormal DNA reprogramming.
667 In SCNT placentas, the promoter regions of *Phlda2* and *Peg10* genes showed
668 hypermethylation, while those of *Igf2r* and *Znf597* genes showed hypomethylation,
669 which were consistent with the changes in accessibility. At the same time, histone
670 modifications also presented a disordered pattern. In SCNT placentas, the levels of
671 H3K4me3 in the promoter regions of *Phlda2* and *Znf597* genes decreased, while the
672 levels of H3K9me3/H3K27me3 increased. In the promoter regions of *Igf2r* and *Znf597*
673 genes, the H3K4me3 modification was enhanced, while the H3K9me3 and H3K27me3
674 modifications were significantly weakened. These abnormal histone modifications
675 further led to abnormal chromatin state and gene expression abnormalities (Figure 5E).
676 The results indicate that there is a close regulatory relationship among DNA
677 methylation, histone modification, and chromatin accessibility, which jointly caused
678 the dysregulation of imprinted gene expression in SCNT placentas. Notably, treatment
679 with Enasidenib mesylate can restore these abnormal epigenetic states to a certain
680 extent, while supplementation with α -KG reversed this effect (Figure 5E). This further
681 confirmed the important role of metabolic-epigenetic cross-regulation in SCNT
682 placental development.

Maternal intake of α -KG leads to hypertrophy of placentas during the middle and late stages of pregnancy

To verify the conserved mechanism of the Idh2- α -KG regulatory axis during mammalian placental development, we established a *M. musculus* model for in-vivo studies (Figure 6A). We systematically evaluated the role of this metabolic pathway through prenatal drinking water intervention (0.75% α -KG and 10 mg/kg Enasidenib mesylate). The results showed that there were no significant differences in the body weights of pregnant *M. musculus* (Supplementary Figure S7A–C). The α -KG treatment group showed a trend of increased placental volume and weight on the 16th day of pregnancy, and the fetal weight also significantly increased on the 19th day of pregnancy (Figure 6B, C; Supplementary Figure S7D, E). This growth-promoting effect was time-dependent. Even if the supplementation of α -KG was stopped on the 10th day of pregnancy, the enlarged placental phenotype could still be maintained. Meanwhile, we found that the Idh2 inhibitor Enasidenib mesylate could significantly antagonize the growth-promoting effect of α -KG, thereby confirming the specific regulatory role of the Idh2- α -KG pathway. Metabolite detection results showed that the changes in α -KG levels in different tissues were highly consistent with the phenotypic changes (Figure 6D; Supplementary Figure S7H).

Further analysis of the placental histological structure by HE section staining showed that the treatment with α -KG significantly increased the area of the placental labyrinth layer and changed its boundary morphology (Figure 6E, F). This indicates that it may affect placental development by promoting cell proliferation or proliferative

remodeling. Such changes in histological structure could be partially reversed by Enasidenib mesylate, indicating that the Idh2- α -KG axis regulates the morphology of placental tissues and influences fetal development. Multi-time-point analysis revealed that the effects of α -KG on the placenta and the fetus vary in terms of developmental timing. The effects of α -KG on the placenta occurred in the early and middle stages of pregnancy, while changes in fetal weight were only observed in the late stage (Supplementary Figure S7D, E). It is noteworthy that this study found that α -KG intervention not only affects placental development but also significantly increases liver weight, suggesting that α -KG may affect the development of multiple organs through systemic metabolic regulation. Similarly, this weight-gain phenomenon can be reversed by Enasidenib mesylate (Supplementary Figure S7F, G).

The metabolite α -KG coordinately regulates *M. musculus* placental development through DNA and histone methylation modifications

To further explore the epigenetic mechanism by which α -KG regulates placental hypertrophy in *M. musculus*, we detected the epigenetic modifications in placental tissues from different treatment groups. We found that the α -KG treatment group showed enhanced DNA demethylation modification on 16th and 19th days of pregnancy, with a significant increase in 5hmc modification. Additionally, characteristic changes in histone modifications were observed, specifically an increase in H3K4me3 modification and a decrease in H3K9me3 and H3K27me3 modifications. Further research showed that these changes in epigenetic modification levels were associated with changes in the expression of key regulatory enzymes, with the expression levels

of DNMT3B, TET3, and KDM6B showing a downward trend (Figure 6G–L). Moreover, when the Idh2 inhibitor Enasidenib mesylate was used in combination, it could significantly reverse the changes in these epigenetic modification levels induced by α -KG and upregulate the expression levels of DNMT3B, TET3, and KDM6B (Figure 6G–L). Notably, even when α -KG supplementation was stopped on the 10th day of pregnancy, these changes in epigenetic modifications could still be maintained in the middle and late stages of pregnancy (the 16th and 19th days), indicating that α -KG can induce persistent epigenetic reprogramming during a specific developmental window. These findings suggest that α -KG can promote placental hypertrophy by dynamically regulating the DNMT3B/TET3/KDM6B expression network and establishing specific DNA and histone methylation modification patterns.

α -KG rescues placental development defects in *Idh2*^{+/-} *M. musculus* by restoring DNA and histone methylation modification levels

Based on our previous findings, we used three groups of pregnant mouse models: WT, *Idh2*^{+/-}, and *Idh2*^{+/-} *M. musculus* supplemented with α -KG, to further explore the regulatory role of Idh2-regulated α -KG in *M. musculus* placental development (Supplementary Figure S8A, B). We did not use the homozygous *Idh2* knockout (*Idh2*^{-/-}) *M. musculus* model. Previous experiments have shown that the embryonic mortality and malformation rates of *Idh2*^{-/-} *M. musculus* are extremely high. The absence of Idh2 disrupts normal metabolism and physiological functions, making it difficult to obtain a sufficient number of viable embryos for statistical analysis. Therefore, we chose the *Idh2*^{+/-} *M. musculus* model to study placental development, avoiding the interference of

high mortality and malformation rates. The experimental results showed that at the 16th day of pregnancy, there were no significant differences in the body weights of the female *M. musculus* in each group (Supplementary Figure S8–E). However, the *Idh2*^{+/-} *M. musculus* exhibited obvious placental development defects. Their placental volume and weight were significantly lower than those of the WT *M. musculus*. Histological structure analysis revealed that the area of the placental labyrinth layer in *Idh2*^{+/-} *M. musculus* was significantly reduced, which might be the cause of the placental development defects (Figure 7A–C). Correspondingly, the fetal weights in the *Idh2*^{+/-} group were also relatively lower (Supplementary Figure S8F, G). The detection results of α -KG content in maternal serum, placenta, and various organs showed that the knockdown of *Idh2* led to a decrease in the α -KG level in maternal serum and various tissues. The supplementation of α -KG in drinking water could restore the decreased α -KG level caused by *Idh2* knockdown (Figure 7D). Additionally, we found that the supplementation of α -KG in drinking water could rescue the placental development defects caused by *Idh2* knockdown, manifested as the increases in placental weight, volume, and the area of the placental labyrinth layer, as well as the increase in fetal weights (Figure 7A–C). Epigenetic modification detection revealed that the placental tissues of *Idh2*^{+/-} *M. musculus* exhibited abnormal epigenetic modification characteristics, including an increase in 5mc modification level, a decrease in 5hmc modification level, a decrease in histone H3K4me3 modification level, and an increase in H3K9me3 and H3K27me3 modification levels (Figure 7E–G). These changes were related to the down-regulation of TET3 and KDM6B expression. Importantly, we found

that the supplementation of α -KG during the feeding of *Idh2*^{+/-} *M. musculus* could reverse this abnormal phenomenon, restoring the epigenetic modification patterns and levels in the placentas of *Idh2*^{+/-} *M. musculus* to a state similar to that of WT *M. musculus*.

DISCUSSION

Since the first application of SCNT technology, a variety of cloned animals have been successfully obtained using this technology (Loi et al., 2016; Matoba et al., 2018a; Ogura et al., 2013). However, the pregnancy success rate of cloned animals remains at a relatively low level. Even if pregnancy is successful, the fetuses often present various abnormalities, such as obesity, immunodeficiency, respiratory diseases, premature death, and excessive placental growth during pregnancy (Ao et al., 2017; Smith et al., 2012; Tanaka et al., 2001). The placenta plays a crucial role during pregnancy. It is the key organ for gas exchange and nutrient exchange between the fetus and the mother, and also serves as an immune barrier (Liu et al., 2018). The normal development of the placenta during pregnancy plays a decisive role in the smooth progress of the entire gestation process (Inoue et al., 2010; Matoba et al., 2014). Therefore, improving the pregnancy success rate of SCNT animals has become a crucial issue that needs to be urgently addressed. In this study, through the integration of multi-omics analysis and functional verification, the core regulatory role of the *Idh2*- α -KG metabolic axis in the abnormal development of SCNT placentas was systematically revealed for the first time.

During the development of the placenta in cloned animals, some significant abnormal phenomena can be observed. These pathological defects mainly manifested

in three aspects: epigenetic disorder, impairment of blood vessel formation, and metabolic dysfunction. Additionally, abnormal expression of imprinted genes has been observed in the placentas of cloned mice and in trophoblast stem cells derived from SCNT (Hirose et al., 2018; Kim et al., 2016; Matoba et al., 2018a; Su et al., 2011; Wang et al., 2020a). In this study, we also observed abnormal epigenetic modifications of imprinted genes in the placentas of cloned *B. taurus*. Consistent with the observations of placental formation, volume density, and placental defects in SCNT *B. taurus* and *Ovis aries* (Chavatte-Palmer et al., 2012; Fletcher et al., 2007), the present study also observed phenomena such as loose chorio-caruncular interdigitation and trophoblast development defects in the placentas of SCNT *B. taurus*. Although the removal of the H3K9me3 epigenetic modification barrier in the donor cells by the Kdm4d demethylase significantly increased the blastocyst formation rate and implantation rate of SCNT *M. musculus* embryos, only less than 15% of the embryos could develop normally to term, and there was a widespread problem of excessive placental growth (Chung et al., 2015; Liu et al., 2016; Matoba et al., 2014). Even with the combination method of *Xist* gene knockout and Kdm4d treatment, the problems of post-implantation developmental arrest and abnormal placental enlargement still cannot be completely resolved (Matoba et al., 2018a). Although the current epigenetic reprogramming methods can partially improve the efficiency of SCNT, they have not completely overcome the key obstacles in the placental development. In this study, it was found that the modification levels of H3K4me3 and 5hmc in SCNT placentas were upregulated, while the H3K9me3 and H3K27me3 were downregulated. This further indicates that there are residual

815 epigenetic barriers in the placentas of SCNT *B. taurus*. Moreover, the abnormal
816 placental hypertrophy induced by α -KG in *M. musculus* also recapitulated the
817 developmental disorders and epigenetic dysregulation observed in SCNT placentas. Of
818 note, the present study found that both 5mC and 5hmC levels were simultaneously
819 increased in SCNT placentas. This observation, seemingly contradictory, actually
820 reflects the disrupted homeostasis between DNA methylation and demethylation in
821 SCNT placentas. As shown in Figure 1L-Q, the expression of both DNMT3B and
822 TET3 was significantly upregulated in SCNT placentas. The elevated
823 methyltransferases promoted de novo 5mC synthesis, whereas TET enzymes, activated
824 by accumulated α -KG, converted part of 5mC into 5hmC, ultimately leading to
825 simultaneous increases in both modifications. Previous studies have demonstrated that
826 TET activation can markedly elevate 5hmC levels while maintaining or even increasing
827 global 5mC levels (Ito et al., 2011). Therefore, the co-elevation of 5mC and 5hmC in
828 SCNT placentas is not conflicting, but represents one of the characteristic
829 manifestations of impaired epigenetic reprogramming. Notably, the abnormal
830 epigenetic modifications in the placentas of SCNT *B. taurus* can be rescued by
831 regulating the expression of *Idh2* to affect the content of α -KG. During SCNT,
832 incomplete nuclear reprogramming and mitochondrial dysfunction jointly drive *Idh2*
833 upregulation and α -KG metabolic imbalance. Specifically, incomplete reprogramming
834 directly causes aberrant epigenetic modifications at the *Idh2* promoter, disrupting its
835 transcription, while inherent mitochondrial dysfunction and impaired energy
836 metabolism indirectly trigger TCA cycle abnormalities and α -KG accumulation. These

metabolic disturbances then disrupt downstream epigenetic modifications and imprinted gene expression, ultimately leading to placental hypertrophy.

As a key intermediate metabolite in TCA cycle, in vitro studies have shown that α -KG plays an important role in maintaining the pluripotency of ESCs and promoting the primitive differentiation of human pluripotent stem cells and germ cells (Lu et al., 2019; TeSlaa et al., 2016). Unlike previous reports that α -KG mainly maintains pluripotency of ESCs by regulating H3K27me3, in this study, we found that the treatment with dm- α -KG showed a broader epigenetic regulatory ability in placental tissues. It significantly changed the expression levels of multiple epigenetic modification markers such as 5hmc, H3K4me3, H3K9me3, and H3K27me3 in bovine placental mesenchymal cells. This finding contrasts sharply with the results of Nerum (2025) et al, in ESCs. This tissue specificity may stem from the unique epigenetic landscape and metabolic characteristics of the placenta. Modifications of histone tails can regulate gene expression by controlling the accessibility of chromatin to transcription factors and RNA polymerases (Eckersley-Maslin et al., 2018; Jambhekar et al., 2019; Schulz et al., 2019). Among them, H3K4me3 promotes chromatin opening by recruiting the transcription initiation complex, H3K27me3 is involved in the formation of facultative heterochromatin, and H3K9me3 mediates heterochromatin formation and inhibits gene expression (Jambhekar et al., 2019). There is a precise coordinated regulatory relationship between these histone modifications and DNA methylation (Danese et al., 2021; Wiekowski et al., 1993). As discovered by Li (2025) et al, in erythropoiesis, the knockdown of Idh1 affected the H3K79me3 modification

by altering the α -KG level, which in turn regulated chromatin accessibility and led to impaired erythropoiesis. KDM6A alters the chromatin structure by regulating the modification levels of H3K27me2 and H3K27me3, and this process can be reversed by α -KG intervention (Schuettengruber et al., 2017; Schwartz et al., 2007; Zhang et al., 2025). In this study, we found that the genomic TSS region of the cloned *B. taurus* placenta exhibited an abnormal chromatin open state, which was closely related to the tissue-specific epigenetic modification profile. However, whether the Idh2- α -KG metabolic axis directly regulates the accessibility of genomic chromatin, and whether this regulation is mediated by specific epigenetic modifications, still need further investigation.

Notably, the expression patterns of DNMT3B, TET3, and KDM6B differed significantly between SCNT *B. taurus* placentas and α -KG-treated *M. musculus* placentas in the present study. These epigenetic modifier genes were significantly upregulated in SCNT *B. taurus* placentas, but markedly downregulated in *M. musculus* placentas following α -KG administration. We speculate that this discrepancy may arise from species-specific regulatory mechanisms, differences in gestational stages and treatment regimens between *B. taurus* and *M. musculus*, as well as compensatory negative feedback responses. *Bos taurus* have a cotyledonary placenta composed of both fetal and maternal components, whereas *M. musculus* possess a discoid placenta formed by the attachment of a round or oval endometrial region to the chorio-allantois, with distinct cellular composition and metabolic characteristics. In terms of treatment, SCNT *B. taurus* placentas represent a pathological state with long-term abnormal

881 accumulation of α -KG during late gestation, whereas *M. musculus* only received short-
882 term α -KG intervention during pregnancy. Sustained elevation of α -KG may trigger
883 compensatory upregulation of epigenetic enzymes in *B. taurus*, whereas short-term α -
884 KG treatment is more likely to induce feedback inhibition after initial activation, which
885 may collectively contribute to the opposite expression trends of DNMT3B, TET3, and
886 KDM6B observed between *B. taurus* and *M. musculus* models. Although the expression
887 levels of epigenetic regulators such as DNMT3B, TET3, and KDM6B showed opposite
888 trends between *B. taurus* and *M. musculus* models, the overall modification patterns of
889 H3K4me3, H3K9me3, and H3K27me3 remained highly consistent. We propose that as
890 a key metabolite, α -KG can directly activate the activity of histone demethylases, and
891 its regulatory effect on histone modifications is stronger than that at the gene
892 transcription level. In addition, the existence of species-specific transcriptional
893 feedback mechanisms may ultimately lead to consistent global modification patterns of
894 H3K4me4, H3K9me3, and H3K27me3 between *B. taurus* and *M. musculus*.

895 Although the placentas of *B. taurus* and *M. musculus* have differences in their
896 anatomical and histological structures, the mammalian placentas still retain some basic
897 biological functions and regulatory mechanisms. Therefore, using a *M. musculus* model
898 to validate the effect of α -KG on placental development is still representative. The
899 present study found that the supplementation of α -KG during pregnancy not only
900 significantly increased the volume and weight of the placenta, but also changed the
901 modification levels of DNA methylation (5mc/5hmc) and histone methylation
902 (H3K9me3/H3K27me3). These changes were already evident in the early stage of

903 pregnancy (day 16), indicating that α -KG may play a role in the initial stage of placental
904 development. In addition, we found that the effects of α -KG could be reversed by
905 inhibiting *Idh2*, confirming the crucial role of the *Idh2*- α -KG metabolic axis in
906 placental development. This finding is consistent with previous research conclusion
907 that α -KG improves stem cell function by regulating the levels of epigenetic
908 modifications (Wang et al., 2020b). In this study, we focused our research on the
909 reproductive process and for the first time confirmed the specific effect of α -KG on
910 placental tissue in an in vivo model. The research of Zhang (2019) et al., focused on the
911 embryonic development stage, and they found that exposure to α -KG from the
912 pronuclear stage to the blastocyst stage could increase the embryonic development rate.
913 Moreover, after transfer of the embryos to the recipient *M. musculus*, the birth weight
914 and weaning weight of the fetuses were increased significantly. Meanwhile, the
915 treatment with α -KG significantly enhanced the activity of TET enzymes and the ratio
916 of 5hmc/5mc. However, this study did not conduct an in-depth investigation on the
917 placenta. This study also confirmed that the supplementation of α -KG significantly
918 increased the fetal weight, and this phenomenon was more obvious in the late stage of
919 pregnancy. Particularly noteworthy is that the placental hypertrophy caused by α -KG,
920 as well as the enlargement of the spongiotrophoblast area and the unclear boundary, are
921 highly similar to the phenotypes of SCNT placentas reported previously (Matoba et al.,
922 2018a; Ogura 2020). This provides a new perspective for us to understand the abnormal
923 development of cloned placentas. In this study, we also found that the biological effects
924 of α -KG exhibit organ synergy. In addition to influencing placental-fetal development,

α -KG supplementation also led to a significant increase in the weight of the maternal liver, indicating that α -KG may coordinate the growth and development of multiple organs through the endocrine or metabolic network. This finding provides a new molecular perspective for understanding metabolic disorders during pregnancy, such as fetal overgrowth and liver dysfunction associated with gestational diabetes. Future research needs to further clarify the specific action mechanisms of α -KG in different organs and its potential clinical translational value.

Although the present study systematically elucidates the regulatory role of the Idh2- α -KG metabolic axis in placental hypertrophy of SCNT *B. taurus*, it is important to acknowledge that our investigations were primarily focused on placental tissues and placental mesenchymal cells. The ultimate goal of improving placental quality is to achieve healthier fetal development and better pregnancy outcomes. However, due to the inherent challenges of the *B. taurus* SCNT model-including the difficulty of constructing SCNT embryos, the long gestational period of *B. taurus*, and the low success rate of pregnancy-systematic evaluation of fetal tissues, fetal development, and overall fetal quality was not completed in the current study. Nevertheless, the placental abnormalities observed in SCNT *B. taurus*, such as trophoblast hyperplasia, impaired epigenetic reprogramming, and dysregulation of imprinted genes, are well-recognized to have profound impacts on fetal growth and postnatal health. Given that α -KG is a key metabolite that can directly influence epigenetic modifications and gene expression, it is plausible that the Idh2- α -KG axis may also exert direct or indirect effects on ICM-derived tissues, including the fetus itself. Future studies should extend the scope of

investigation to systematically assess fetal development, organ formation, and long-term health outcomes in SCNT offspring, thereby clarifying how the Idh2- α -KG axis affects overall SCNT model outcomes beyond the placenta. Such efforts will be essential for translating epigenetic and metabolic interventions into practical strategies to improve cloning efficiency and offspring health.

However, this study still has certain limitations. Firstly, the systemic metabolic effects of α -KG may indirectly affect fetal development through non-placental pathways. Secondly, the unique histological structure of the *B. taurus* placenta may affect the efficiency of metabolite trans-placental transport. Although the *M. musculus* model used in this study validated the conserved regulatory role of the Idh2- α -KG axis in placental development, the inherent anatomical and physiological differences between *M. musculus* and *B. taurus* placentas (discoid placenta in *M. musculus* versus cotyledonary placenta in *B. taurus*). In the present study, we primarily assessed morphological, histological, and epigenetic modifications in *M. musculus* placentas, but did not systematically evaluate functional parameters such as nutrient transport efficiency, hormone secretion function, or blood perfusion. Therefore, whether α -KG-induced placental hypertrophy in *M. musculus* fully recapitulates the functional abnormalities observed in SCNT *B. taurus* placentas remains to be further investigated. Future studies combining placental perfusion assays, nutrient transport measurements, and endocrine function assessments will help to more comprehensively elucidate the effects of α -KG on placental function and its cross-species conservation. Finally, the quantitative relationship between the α -KG concentration gradient and epigenetic

modifications has not been fully elucidated. Furthermore, this study was mainly conducted using placental tissues and placental mesenchymal cells, without in-depth dissection of the subtype-specific regulation in distinct placental cell lineages such as trophoblasts and endothelial cells. Future studies could combine single-cell sequencing and related techniques to further clarify the cell-type-specific roles of α -KG in the placenta. Meanwhile, although IDH2 inhibitors have been clinically applied, primarily for the treatment of IDH2-mutant acute myeloid leukemia (Stein et al., 2017), their biosafety in reproductive applications still requires systematic evaluation. More comprehensive in vivo safety studies should be performed in the future to investigate their potential impacts on fetal development and support their translational application. As a key mitochondrial enzyme, IDH2 is essential not only for α -KG generation but also for maintaining mitochondrial redox homeostasis (NADP⁺/NADPH) and antioxidant capacity. However, this study focused on the epigenetic regulatory role of the Idh2- α -KG axis, without investigating its direct effects on mitochondrial function and redox status. Mitochondrial dysfunction may also contribute to the abnormal development of SCNT *B. taurus* placentas. Future studies combining mitochondrial functional assays and redox detection will help further dissect the synergistic network of IDH2 in metabolism-redox balance-epigenetics. In subsequent studies, the use of conditional gene knockout and single-cell multi-omics analysis will help to more precisely decipher these complex regulatory networks. Future studies should also combine mitochondrial functional assays and redox index detection to further dissect the synergistic regulatory network of IDH2 in terms of “metabolism-redox balance-

epigenetics.”

Despite these limitations, the core finding of this study can be integrated into a coherent mechanistic model. Based on the multi-omics and functional findings of this study, we propose a schematic model illustrating the regulatory mechanism of the *Idh2*- α -KG axis in SCNT *B. taurus* placental hypertrophy (Figure 8). As shown in the diagram, in SCNT *B. taurus* placentas and derived bPMSCs, *Idh2* expression is significantly upregulated, thereby enhancing the conversion of isocitrate to α -KG. The elevated α -KG levels further activate TET3 and multiple lysine demethylases (KDM4B, KDM5B, KDM6B), leading to increased levels of 5hmC and H3K4me3, as well as decreased levels of H3K9me3 and H3K27me3. These epigenetic alterations affect the chromatin accessibility of key imprinted genes, thereby regulating their expression levels and ultimately resulting in placental hypertrophy. In contrast, CON placentas maintain normal α -KG levels and a balanced epigenetic modification state.

In summary, this study revealed the abnormal metabolic mechanism of SCNT placenta development, and established a regulatory axis of metabolism-epigenetic modification-placenta development. Our findings indicate that inhibiting *Idh2* activity may serve as a novel strategy to improve cloning efficiency in livestock and alleviate abnormal placental development such as placental hypertrophy, providing new insights for enhancing the application of SCNT technology in animal production. Furthermore, the association between *Idh2*/ α -KG metabolic imbalance and placental dysfunction revealed in this study offers a valuable reference for exploring the pathogenesis of human abnormal placental development-related diseases, further broadening the

scientific significance of this research. This work provides a new theoretical basis and potential intervention strategies for improving animal cloning efficiency and understanding human placental diseases. These findings expand our understanding of the role of metabolic reprogramming in reproductive development and hold significant scientific and practical value.

DATA AVAILABILITY

All relevant data are available within the article and its supplementary information. The raw RNA-seq data analyzed in this study are available from NCBI database (BioProjectID PRJNA1177607), China National Center for Bioinformation database of Genome Sequence Archive (GSA <https://ngdc.cncb.ac.cn/gsa>) (CRA014398), and Science Data Bank database (doi: 10.57760/sciencedb.j00139.00114). The raw ATAC-seq data analyzed in this study are available from NCBI database (BioProjectID PRJNA1333875), GSA (PRJCA065385), and Science Data Bank database (doi: 10.57760/sciencedb.j00139.00338). The raw WGBS-seq data of CON and SCNT *B. taurus* placentomes analyzed in this study can be obtained from the NCBI database (BioProject ID PRJNA1334340), GSA (PRJCA065387), and Science Data Bank database (doi: 10.57760/sciencedb.38633). The raw WGBS-seq data of *B. taurus* placental mesenchymal cells that regulate the content of α -KG analyzed in this study can be obtained from the NCBI database (BioProject ID PRJNA1334612), GSA (PRJCA065579), and Science Data Bank database (doi: 10.57760/sciencedb.38694). The raw CHIP-seq data of *B. taurus* placental mesenchymal cells that regulate the content of α -KG analyzed in this study can be obtained from the NCBI database

(BioProject ID PRJNA1335187), GSA (PRJCA065644), and Science Data Bank database (doi: 10.57760/sciencedb.38680). The raw LC-MC metabolomic data analyzed in this study are available from the China National Center for Bioinformatics OMIX database (OMIX ID: OMIX017403).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

G.P.L., and G.H.S., conceived the project; X.Y.Z., Y.Y., H.Y.L., S.Y.T., S.S.W., Y.X.G., and C.J.B., performed the experiments and analyzed the data; L.Y., X.F.L., L.S.S., and C.L.B., contributed to the bioinformatics analysis; X.Y.Z., Y.Y., and G.H.S., wrote the manuscript; X.Y.Z., G.P.L., and G.H.S., contributed to the manuscript revision and discussion. All the authors read the manuscript and approved the final manuscript.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (32560808), the Natural Science Foundation of Inner Mongolia (2024MSS03001), the Program for Young Talents of Science and Technology in Universities of Inner Mongolia Autonomous Region (NJYT23090), the Inner Mongolia Autonomous Region “Challenge-Based Project” (2022JSGS0018).

REFERENCES



- 1057 Agger K, Cloos PA, Christensen J, et al. 2007. UTX and JMJD3 are histone H3K27
1058 demethylases involved in HOX gene regulation and development. *Nature*,
1059 **449**(7163): 731–734.
- 1060 Aldoretta PW, Hay WW, Jr. 1999. Effect of glucose supply on ovine uteroplacental
1061 glucose metabolism. *Am J Physiol*, **277**(4): R947–958.
- 1062 Ao Z, Liu D, Zhao C, et al. 2017. Birth weight, umbilical and placental traits in relation
1063 to neonatal loss in cloned pigs. *Placenta*, **57**: 94–101.
- 1064 Aplin JD, Myers JE, Timms K, et al. 2020. Tracking placental development in health
1065 and disease. *Nat Rev Endocrinol*, **16**(9): 479–494.
- 1066 Arnold DR, Gaspar RC, da Rocha CV, et al. 2017. Nuclear transfer alters placental gene
1067 expression and associated histone modifications of the placental-specific
1068 imprinted gene pleckstrin homology-like domain, family A, member 2 (PHLDA2)
1069 in cattle. *Reprod Fertil Dev*, **29**(3): 458–467.
- 1070 Burton GJ, Fowden AL, Thornburg KL. 2016. Placental Origins of Chronic Disease.
1071 *Physiol Rev*, **96**(4): 1509–1565.
- 1072 Burton GJ, Redman CW, Roberts JM, et al. 2019. Pre-eclampsia: pathophysiology and
1073 clinical implications. *Bmj*, **366**: l2381.
- 1074 Carey BW, Finley LW, Cross JR, et al. 2015. Intracellular α -ketoglutarate maintains
1075 the pluripotency of embryonic stem cells. *Nature*, **518**(7539): 413–416.
- 1076 Chavatte-Palmer P, Camous S, Jammes H, et al. 2012. Review: Placental perturbations
1077 induce the developmental abnormalities often observed in bovine somatic cell
1078 nuclear transfer. *Placenta*, **33**: S99–S104.

1079 Chin RM, Fu X, Pai MY, et al. 2014. The metabolite α -ketoglutarate extends lifespan
 1080 by inhibiting ATP synthase and TOR. *Nature*, **510**(7505): 397–401.

1081 Chung YG, Matoba S, Liu Y, et al. 2015. Histone Demethylase Expression Enhances
 1082 Human Somatic Cell Nuclear Transfer Efficiency and Promotes Derivation of
 1083 Pluripotent Stem Cells. *Cell Stem Cell*, **17**(6): 758–766.

1084 Constant F, Guillomot M, Heyman Y, et al. 2006. Large offspring or large placenta
 1085 syndrome? Morphometric analysis of late gestation bovine placentomes from
 1086 somatic nuclear transfer pregnancies complicated by hydrallantois. *Biol Reprod*,
 1087 **75**(1): 122–130.

1088 Danese A, Richter ML, Chaichoompu K, et al. 2021. EpiScanpy: integrated single-cell
 1089 epigenomic analysis. *Nat Commun*, **12**(1):5228.

1090 Dang L, Yen K, Attar EC. 2016. IDH mutations in cancer and progress toward
 1091 development of targeted therapeutics. *Ann Oncol*, **27**(4): 599–608.

1092 de Botton S, Montesinos P, Schuh AC, et al. 2023. Enasidenib vs conventional care in
 1093 older patients with late-stage mutant-IDH2 relapsed/refractory AML: a
 1094 randomized phase 3 trial. *Blood*, **141**(2): 156–167.

1095 Eckersley-Maslin MA, Alda-Catalinas C, Reik W. 2018. Dynamics of the epigenetic
 1096 landscape during the maternal-to-zygotic transition. *Nat Rev Mol Cell Biol*, **19**(7):
 1097 436–450.

1098 Fletcher CJ, Roberts CT, Hartwich KM, et al. 2007. Somatic cell nuclear transfer in the
 1099 sheep induces placental defects that likely precede fetal demise. *Reproduction*,
 1100 **133**(1): 243–255.

1101 Fowden AL, Coan PM, Angiolini E, et al. 2011. Imprinted genes and the epigenetic
 1102 regulation of placental phenotype. *Prog Biophys Mol Biol*, **106**(1): 281–288.

1103 Gao CW, Lin W, Riddle RC, et al. 2023. Novel mouse model of Weaver syndrome
 1104 displays overgrowth and excess osteogenesis reversible with KDM6A/6B
 1105 inhibition. *bioRxiv*, **06**: 546270. Doi: 10.1101/2023.06.23.546270.

1106 Gao R, Wang C, Gao Y, et al. 2018. Inhibition of Aberrant DNA Re-methylation
 1107 Improves Post-implantation Development of Somatic Cell Nuclear Transfer
 1108 Embryos. *Cell Stem Cell*, **23**(3): 426–435.

1109 Han SJ, Jang HS, Noh MR, et al. 2017. Mitochondrial NADP(+)-Dependent Isocitrate
 1110 Dehydrogenase Deficiency Exacerbates Mitochondrial and Cell Damage after
 1111 Kidney Ischemia-Reperfusion Injury. *J Am Soc Nephrol*, **28**(4): 1200–1215.

1112 Hirose M, Hada M, Kamimura S, et al. 2018. Aberrant imprinting in mouse trophoblast
 1113 stem cells established from somatic cell nuclear transfer-derived embryos.
 1114 *Epigenetics*, **13**(7): 693–703.

1115 Huang F, Luo X, Ou Y, et al. 2023. Control of histone demethylation by nuclear-
 1116 localized α -ketoglutarate dehydrogenase. *Science*, **381**(6654): eadf8822.

1117 Inoue K, Hirose M, Inoue H, et al. 2017. The Rodent-Specific MicroRNA Cluster
 1118 within the Sfmt2 Gene Is Imprinted and Essential for Placental Development.
 1119 *Cell Rep*, **19**(5): 949–956.

1120 Inoue K, Kohda T, Sugimoto M, et al. 2010. Impeding Xist expression from the active
 1121 X chromosome improves mouse somatic cell nuclear transfer. *Science*, **330**(6003):
 1122 496–499.

- 1123 Ispada J, da Fonseca Junior AM, de Lima CB, et al. 2020. Tricarboxylic Acid Cycle
1124 Metabolites as Mediators of DNA Methylation Reprogramming in Bovine
1125 Preimplantation Embryos. *Int J Mol Sci*, **21**(18): 6868.
- 1126 Ito S, Shen L, Dai Q, et al. 2011. Tet proteins can convert 5-methylcytosine to 5-
1127 formylcytosine and 5-carboxylcytosine. *Science*, **333**(6047):n1300–n1303.
- 1128 Jambhekar A, Dhall A, Shi Y. 2019. Roles and regulation of histone methylation in
1129 animal development. *Nat Rev Mol Cell Biol*, **20**(10): 625–641.
- 1130 Jo SH, Son MK, Koh HJ, et al. 2001. Control of mitochondrial redox balance and
1131 cellular defense against oxidative damage by mitochondrial NADP⁺-dependent
1132 isocitrate dehydrogenase. *J Biol Chem*, **276**(19): 16168–16176.
- 1133 Kang HS, Lee JH, Oh KJ, et al. 2020. IDH1-dependent α -KG regulates brown fat
1134 differentiation and function by modulating histone methylation. *Metabolism*, **105**:
1135 154173.
- 1136 Kim HR, Lee JE, Oqani RK, et al. 2016. Aberrant Expression of TIMP-2 and PBEF
1137 Genes in the Placentae of Cloned Mice Due to Epigenetic Reprogramming Error.
1138 *PLoS One*. **5**(11): e0166241.
- 1139 Kishigami S, Mizutani E, Ohta H, et al. 2006. Significant improvement of mouse
1140 cloning technique by treatment with trichostatin A after somatic nuclear transfer.
1141 *Biochem Biophys Res Commun*, **340**(1): 183–189.
- 1142 Koivunen P, Hirsilä M, Remes AM, et al. 2007. Inhibition of hypoxia-inducible factor
1143 (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell
1144 metabolism and stabilization of HIF. *J Biol Chem*, **282**(7): 4524–4532.

- 1145 Li J, He Y, Tan Z, et al. 2018. Wild-type IDH2 promotes the Warburg effect and tumor
1146 growth through HIF1 α in lung cancer. *Theranostics*, **8**(15): 4050–4061.
- 1147 Li M, Zhang H, Wu X, et al. 2025. IDH1 regulates human erythropoiesis by eliciting
1148 chromatin state reprogramming. *Elife*, **13****10**: 100406.
- 1149 Liu J, Liang X, Zhu J, et al. 2008. Aberrant DNA methylation in 5' regions of DNA
1150 methyltransferase genes in aborted bovine clones. *J Genet Genomics*, **35**(9): 559–
1151 568.
- 1152 Liu W, Liu X, Wang C, et al. 2016. Identification of key factors conquering
1153 developmental arrest of somatic cell cloned embryos by combining embryo biopsy
1154 and single-cell sequencing. *Cell Discov*, **2**: 16010.
- 1155 Liu Y, Fan X, Wang R, et al. 2018. Single-cell RNA-seq reveals the diversity of
1156 trophoblast subtypes and patterns of differentiation in the human placenta. *Cell*
1157 *Res*, **28**(8): 819–832.
- 1158 Loi P, Iuso D, Czernik M, et al. 2016. A New, Dynamic Era for Somatic Cell Nuclear
1159 Transfer. *Trends Biotechnol*, **34**(10): 791–797.
- 1160 Lu V, Teitell MA. 2019. Alpha-ketoglutarate: a "magic" metabolite in early germ cell
1161 development. *Embo j*, **38**(1): 0615.
- 1162 Matoba S, Liu Y, Lu F, et al. 2014. Embryonic development following somatic cell
1163 nuclear transfer impeded by persisting histone methylation. *Cell*, **159**(4): 884–895.
- 1164 Matoba S, Nakamuta S, Miura K, et al. 2019. Paternal knockout of Slc38a4/SNAT4
1165 causes placental hypoplasia associated with intrauterine growth restriction in mice.
1166 *Proc Natl Acad Sci U S A*, **116**(42): 21047–21053.

- 1167 Matoba S, Wang H, Jiang L, et al. 2018a. Loss of H3K27me3 Imprinting in Somatic
1168 Cell Nuclear Transfer Embryos Disrupts Post-Implantation Development. *Cell*
1169 *Stem Cell*, **23**(3): 343–354.
- 1170 Matoba S, Zhang Y. 2018b. Somatic Cell Nuclear Transfer Reprogramming:
1171 Mechanisms and Applications. *Cell Stem Cell*, **23**(4): 471–485.
- 1172 Ogura A. 2020. How to improve mouse cloning. *Theriogenology*, **150**: 215–220.
- 1173 Ogura A, Inoue K, Wakayama T. 2013. Recent advancements in cloning by somatic
1174 cell nuclear transfer. *Philos Trans R Soc Lond B Biol Sci*, **368**(1609): 20110329.
- 1175 Rasmussen KD, Helin K. 2016. Role of TET enzymes in DNA methylation,
1176 development, and cancer. *Genes Dev*, **30**(7): 733–750.
- 1177 Reid MA, Dai Z, Locasale JW. 2017. The impact of cellular metabolism on chromatin
1178 dynamics and epigenetics. *Nat Cell Biol*, **19**(11): 1298–1306.
- 1179 Scagliotti V, Costa Fernandes Esse R, Willis TL, et al. 2021. Dynamic Expression of
1180 Imprinted Genes in the Developing and Postnatal Pituitary Gland. *Genes (Basel)*,
1181 **12**(4): 0509.
- 1182 Schuettengruber B, Bourbon HM, Di Croce L, et al. 2017. Genome Regulation by
1183 Polycomb and Trithorax: 70 Years and Counting. *Cell*, **171**(1): 34–57.
- 1184 Schulz KN, Harrison MM. 2019. Mechanisms regulating zygotic genome activation.
1185 *Nat Rev Genet*, **20**(4): 221–234.
- 1186 Schwartz YB, Pirrotta V. 2007. Polycomb silencing mechanisms and the management
1187 of genomic programmes. *Nat Rev Genet*, **8**(1): 9–22.
- 1188 Si X, Shao M, Teng X, et al. 2024. Mitochondrial isocitrate dehydrogenase impedes



1189 CAR T cell function by restraining antioxidant metabolism and histone acetylation.
 1190 *Cell Metab*, **36**(1): 176–192.

1191 Silveira MM, Vargas LN, Bayão HXS, et al. 2019. DNA methylation of the endogenous
 1192 retrovirus Fematrin-1 in fetal placenta is associated with survival rate of cloned
 1193 calves. *Placenta*, **88**: 52–60.

1194 Smith LC, Suzuki J, Jr., Goff AK, et al. 2012. Developmental and epigenetic anomalies
 1195 in cloned cattle. *Reprod Domest Anim*, **4**: 107–114.

1196 Stein EM, DiNardo CD, Pollyea DA, et al. 2017. Enasidenib in mutant IDH2 relapsed
 1197 or refractory acute myeloid leukemia. *Blood*, **130**(6): 722–731.

1198 Su JM, Yang B, Wang YS, et al. 2011. Expression and methylation status of imprinted
 1199 genes in placentas of deceased and live cloned transgenic calves. *Theriogenology*,
 1200 **75**(7): 1346–1359.

1201 Tanaka S, Oda M, Toyoshima Y, et al. 2001. Placentomegaly in cloned mouse concepti
 1202 caused by expansion of the spongiotrophoblast layer. *Biol Reprod*, **65**(6): 1813–
 1203 1821.

1204 TeSlaa T, Chaikovsky AC, Lipchina I, et al. 2016. α -Ketoglutarate Accelerates the
 1205 Initial Differentiation of Primed Human Pluripotent Stem Cells. *Cell Metab*, **24**(3):
 1206 485–493..

1207 Tischler J, Gruhn WH, Reid J, et al. 2019. Metabolic regulation of pluripotency and
 1208 germ cell fate through α -ketoglutarate. *Embo j*, **38**(1): 201899518.

1209 Van Nerum K, Wenzel A, Argemi-Muntadas L, et al. 2025. α -Ketoglutarate promotes
 1210 trophectoderm induction and maturation from naive human embryonic stem cells.

- 1211 *Nat Cell Biol*, **27**(5): 749–761.
- 1212 Wang H, Xiong Q, He G, et al. 2023. Hepatic IDH2 regulates glycolysis and
1213 gluconeogenesis. *Metabolism*, **143**: 155559.
- 1214 Wang LY, Li ZK, Wang LB, et al. 2020a. Overcoming Intrinsic H3K27me3 Imprinting
1215 Barriers Improves Post-implantation Development after Somatic Cell Nuclear
1216 Transfer. *Cell Stem Cell*, **27**(2): 315–325.
- 1217 Wang Y, Deng P, Liu Y, et al. 2020b. Alpha-ketoglutarate ameliorates age-related
1218 osteoporosis via regulating histone methylations. *Nat Commun*, **11**(1): 5596.
- 1219 Wiekowski M, Miranda M, DePamphilis ML. 1993. Requirements for promoter
1220 activity in mouse oocytes and embryos distinguish paternal pronuclei from
1221 maternal and zygotic nuclei. *Dev Biol*, **159**(1): 366–378.
- 1222 Wilmut I, Schnieke AE, McWhir J, et al. 1997. Viable offspring derived from fetal and
1223 adult mammalian cells. *Nature*, **385**(6619): 810–813.
- 1224 Wu SS, Zhao XY, Yang L, et al. Transcription coactivator YAP1 promotes
1225 CCND1/CDK6 expression, stimulating cell proliferation in cloned cattle placentas.
1226 *Zool Res*, **46**(1): 122–138.
- 1227 Wu X, Zhang Y. 2017. TET-mediated active DNA demethylation: mechanism, function
1228 and beyond. *Nat Rev Genet*, **18**(9): 517–534.
- 1229 Xie Z, Zhang W, Zhang Y. 2022. Loss of Slc38a4 imprinting is a major cause of mouse
1230 placenta hyperplasia in somatic cell nuclear transferred embryos at late gestation.
1231 *Cell Rep*, **38**(8): 110407.
- 1232 Xing B, Xu Y, Cheng Y, et al. 2007. Overexpression of IGF2R and IGF1R mRNA in

1233 SCNT-produced goats survived to adulthood. *J Genet Genomics*, **34**(8): 709–719.

1234 Yu X, Wu H, Su J, et al. 2024. Acetyl-CoA metabolism maintains histone acetylation
 1235 for syncytialization of human placental trophoblast stem cells. *Cell Stem Cell*,
 1236 **31**(9): 1280–1297.

1237 Zhang D, Wang Y, Shi Z, et al. 2015. Metabolic reprogramming of cancer-associated
 1238 fibroblasts by IDH3 α downregulation. *Cell Rep*, **10**(8): 1335–1348.

1239 Zhang J, Xiang S, Liu D, et al. 2025. KDM6A downregulation promotes tumor-prone
 1240 cytokines expression in cancer-associated fibroblasts by activating enhancers. *Cell*
 1241 *Death Dis*, **16**(1): 523.

1242 Zhang Z, He C, Zhang L, et al. 2019. Alpha-ketoglutarate affects murine embryo
 1243 development through metabolic and epigenetic modulations. *Reproduction*,
 1244 **158**(2): 123–133.

1245

FIGURE LEGENDS

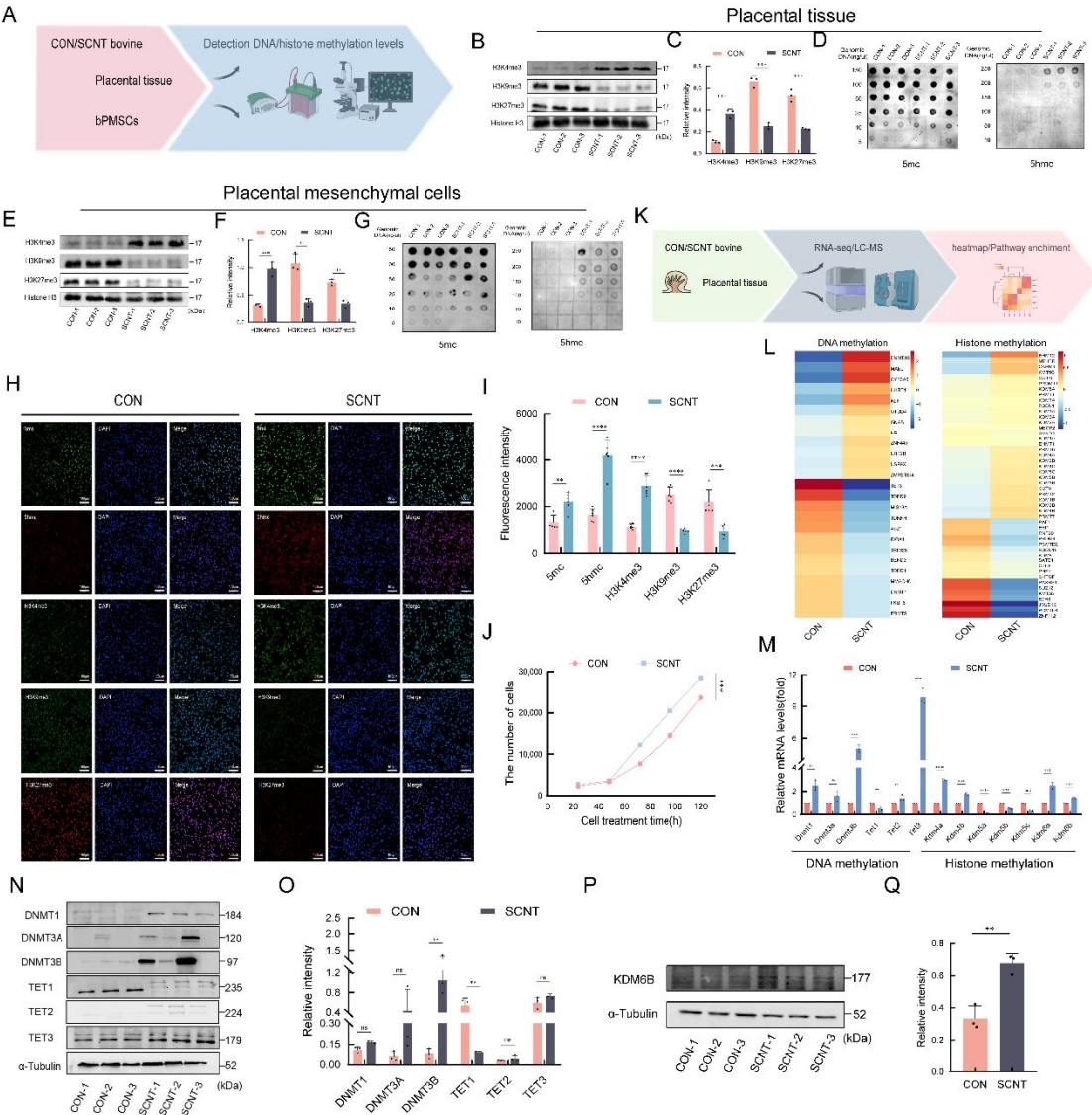


Figure 1 Epigenetic modification patterns of abnormal placentas in SCNT B.

taurus

A: Flow chart of the experimental design. B, C: Immunoblots (left) and analysis of relative protein levels (right) of H3K4me3, H3K9me3, and H3K27me3 in placentomes. Histone H3 was used as an internal reference ($n=3$ biologically independent replicates). D: Dot blot results of 5mc and 5hmc in placentomes ($n=3$ biologically independent

replicates). E, F: Immunoblots (left) and relative protein levels (right) of histone
 methylation in placental mesenchymal cells. Histone H3 was used as a loading control
 ($n=3$ biologically independent replicates). G: Dot blot of 5mc and 5hmc in placental
 mesenchymal cells ($n=3$ biologically independent replicates). H, I:
 Immunofluorescence staining and intensity analysis of epigenetic modifications in
 placental mesenchymal cells. 5mc, H3K4me3, and H3K9me3 were stained green, 5hmc
 and H3K27me3 were stained magenta, and DAPI was stained blue ($n=3$ biologically
 independent replicates). The scale bar is 100 μm . J: CCK-8 assay to detect the
 proliferation ability of placental mesenchymal cells. K: Schematic diagram of the multi-
 omics analysis strategy. L: Heatmap of differentially expressed genes in DNA
 methylation and histone methylation. M: RT-qPCR was used to detect the expression
 levels of DNA methyltransferases and histone methyltransferases in placentomes ($n=3$
 biologically independent replicates). N-Q: Representative images and relative protein
 levels of DNMT1, DNMT3A, DNMT3B, TET1, TET2, TET3, and KDM6B in
 placentomes. α -Tubulin was used as an internal reference ($n=3$ biologically
 independent replicates). Different significance levels are indicated by asterisks (**:
 $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.

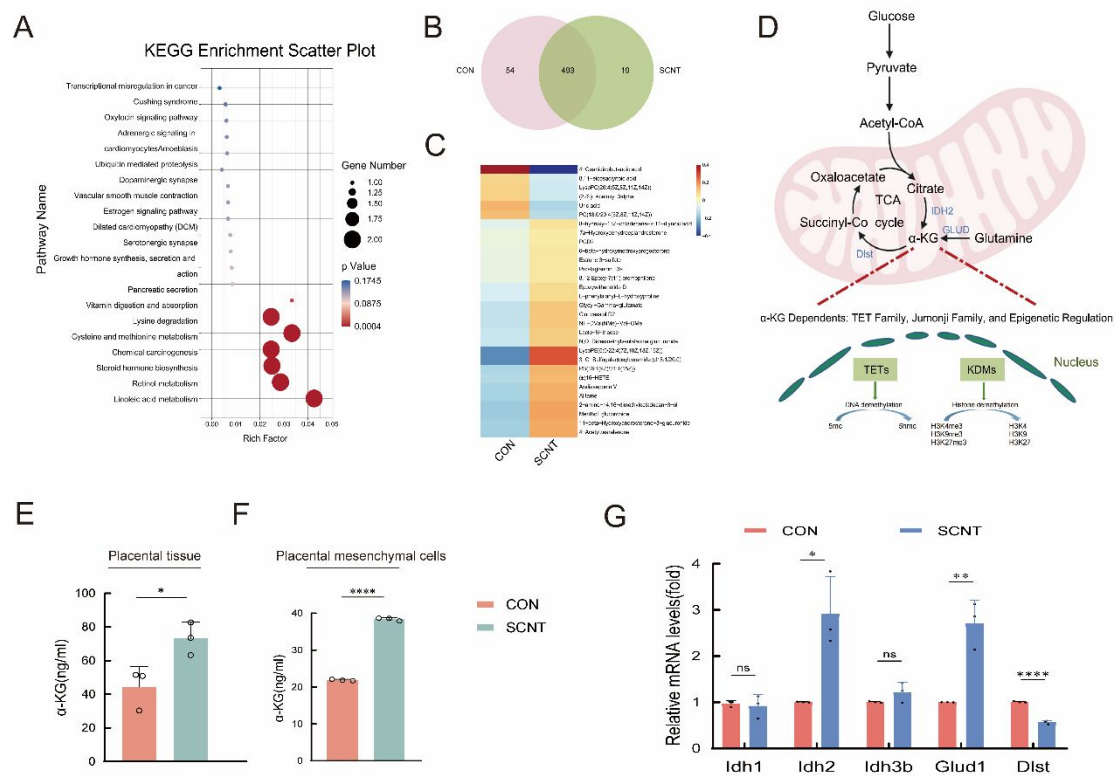
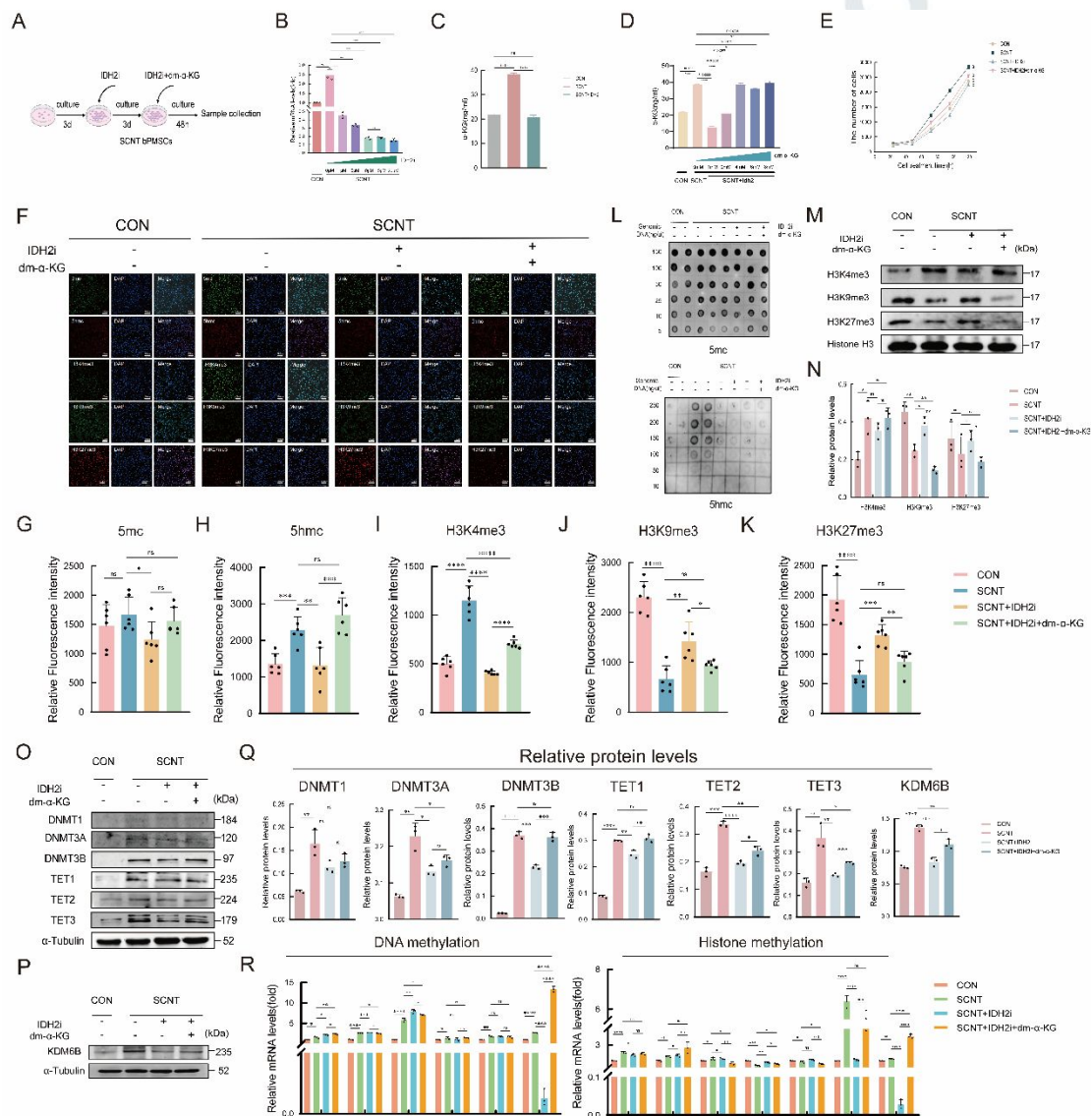


Figure 2 The placentas of **SCNT *B. taurus*** exhibit a high level of α -KG, and the synthetase *Idh2* is highly expressed

A: KEGG enrichment analysis of differentially expressed DNA and histone methylation genes in placentomes of **CON and SCNT *B. taurus***. The P-value is obtained through hypergeometric distribution (two-tailed) and subsequently adjusted using FDR correction. B: Differential metabolites in placentomes of **CON and SCNT *B. taurus*** detected by LC-MS in positive ion mode. A total of 493 differential metabolites were co-expressed between the two groups. C: Cluster analysis of differential metabolites in placentomes of **CON and SCNT *B. taurus*** in positive ion mode. D: Schematic diagram of the participation of α -KG in the TCA cycle and the regulation of epigenetic modifications. E: Levels of α -KG in placentomes of **CON and SCNT *B. taurus*** ($n=3$ biologically independent replicates). F: Levels of α -KG in placental mesenchymal cells

1286 of CON and SCNT *B. taurus* ($n=3$ biologically independent replicates). G: Expression
 1287 levels of α -KG synthesis-related enzymes Idh1, Idh2, Idh3b, Glud1, and Dlst in
 1288 placentomes of CON and SCNT *B. taurus* detected by RT-qPCR ($n=3$ biologically
 1289 independent replicates). Different significance levels are indicated by asterisks (*:
 1290 $P<0.05$; **: $P<0.01$; ****: $P<0.0001$) and ns: Not significant.

1291



1292

1293 **Figure 3 The Idh2- α -KG axis regulates epigenetic reprogramming in the placentas**

1294 **of SCNT *B. taurus***

A: Treatment process of α -KG supplementation (4 mmol/L) and Idh2 inhibitor (Enasidenib mesylate, 10 μ mol/L). B: Concentration screening of Enasidenib mesylate (dose gradient: 0-20 μ mol/L, $n=3$ biologically repeated experiments). C: Inhibitory effect of Enasidenib mesylate (10 μ mol/L) treatment on the α -KG level in cells. D: Concentration screening of α -KG (gradient: 0-8 mmol/L, and 4 mmol/L was finally selected for subsequent experiments, $n=3$ biologically repeated experiments). E: Detection of cell proliferation in different treatment groups by CCK-8 assay. F-K: Analysis of epigenetic markers by immunofluorescence staining. 5mc, H3K4me3, and H3K9me3 were stained green, 5hmc and H3K27me3 were stained magenta, and DAPI was stained blue ($n=3$). The scale bar is 100 μ m. L: Analysis of 5mc and 5hmc by dot blot ($n=3$). M, N: Representative images and relative protein levels of histone modifications detected by Western blot. Histone H3 was used as an internal reference ($n=3$). O-Q: Representative bands (upper) and relative protein levels (lower) of DNMT1, DNMT3A, DNMT3B, TET1, TET2, TET3, and KDM6B. α -Tubulin was used as an internal reference ($n=3$). R: Detection of the expression levels of DNA methyltransferases and histone methyltransferases by RT-qPCR ($n=3$ biologically independent replicates). Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.

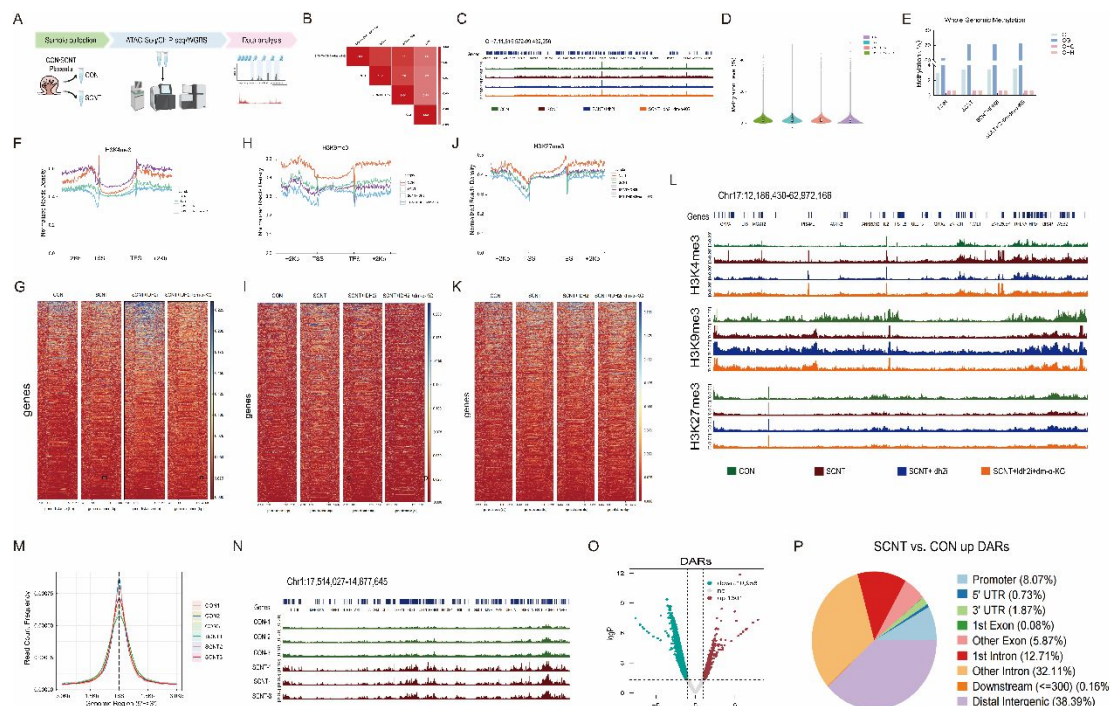


Figure 4 Multi-omics reveals the effect of α -KG on epigenetic modifications in SCNT and the unique chromatin openness characteristics of the placentas of SCNT *B. taurus*

A: Schematic diagram of the experimental design: Analyzing the effect of α -KG on the genomic methylation modification level through WGBS and ChIP-Seq. B: Pearson correlation analysis between samples based on WGBS data. C: IGV visualization of the whole-genome methylation modification level. D: Violin plot of the whole-genome methylation level distribution. E: Proportional distribution of whole-genome methylated C, CG, CHG, and CHH in bovine placental mesenchymal cells after α -KG supplementation and Enasidenib mesylate interference. F, G: ChIP-Seq detection of the distribution of H3K4me3 histone modification across the whole genome. H, I: ChIP-Seq detection of the distribution of H3K9me3 histone modification across the whole genome. J, K: ChIP-Seq detection of the distribution of H3K27me3 histone

modification across the whole genome. L: Epigenetic modification map of chromosome 17 (H3K4me3/H3K9me3/H3K27me3). M: Visualization of ATAC-seq signals covering the entire genome centered on the TSS ($n=3$ biologically independent replicates). N: Standardized ATAC-seq reads across the whole genome. O: Volcano plot of DARs between CON and SCNT *B. taurus* placentomes. P: Genomic distribution of up-regulated differentially accessible regions between CON and SCNT *B. taurus* placentomes. It should be noted that WGBS cannot distinguish 5mC from 5hmC, and the identified methylation levels represent the combined signal of these two modifications.

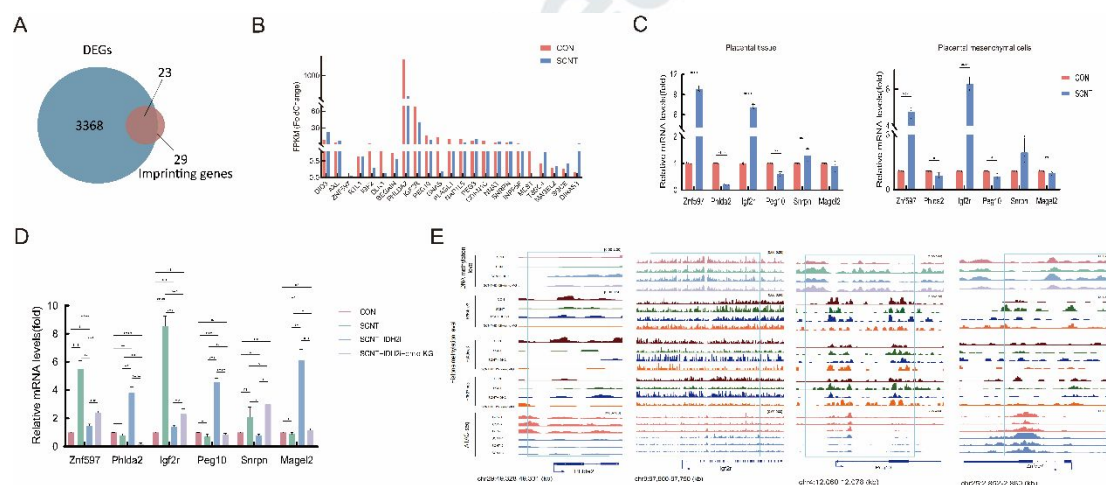


Figure 5 Mechanistic study of the effect of α -KG on the expression of imprinted genes in SCNT placentas

A: A Venn diagram shows the number of imprinted genes among the differentially expressed genes in SCNT *B. taurus* placentomes. B: FPKM expression levels of paternal imprinting and maternal imprinting. C: Detection of the expression levels of placental development-related imprinted genes in CON and SCNT *B. taurus*

placentomes and mesenchymal cells by RT-qPCR ($n=3$ independent biological replicates). D: Detection of the expression levels of imprinted genes in placental mesenchymal cells after α -KG supplementation and Enasidenib mesylate interference by RT-qPCR ($n=3$ independent biological replicates). E: Epigenetic maps and chromatin openness of Phlda2, Igf2r, Peg10, and Znf597. Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.

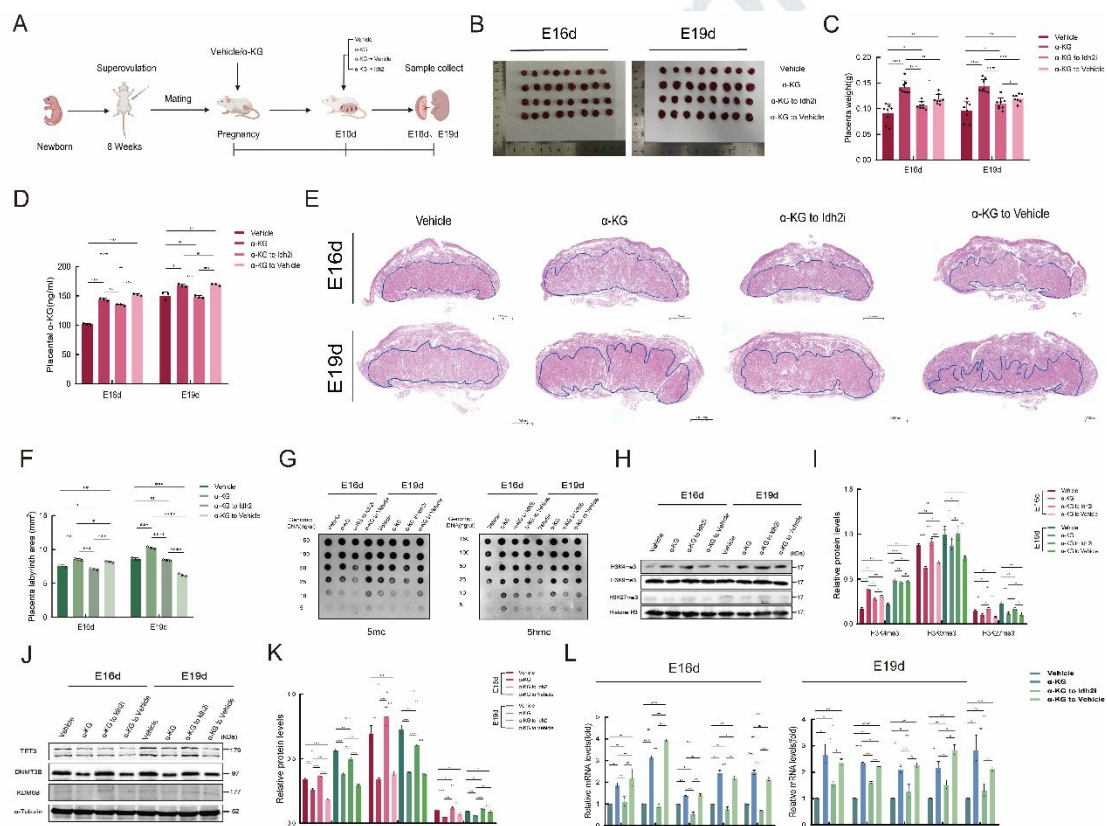


Figure 6 Supplementation of α -KG and drug intervention during pregnancy regulate abnormal placental hyperplasia through epigenetic modifications

A: Flowchart of the experimental design for α -KG supplementation and Enasidenib mesylate intervention during mouse pregnancy. B, C: Schematic diagrams of E16 and

E19 *M. musculus* placentas and placental weights. Placental weights were calculated as the mean value per litter, and data represent mean±SEM. (data from 8 litters of independent biological replicates in each group, repeated 3 times in total). D: Detection of α -KG levels in placentas of different groups at E16 and E19 (pooled samples, $n=3$ independent biological replicates). E, F: HE staining images of placental tissues and quantitative analysis of the area of the labyrinth layer (dashed area). G: Dot blot analysis of whole-genome 5mc and 5hmc levels in placentas. ($n=3$ independent biological replicates). H, I: Western blot detection of histone modification markers H3K4me3, H3K9me3, and H3K27me3 and statistical analysis of their relative expression levels, using Histone H3 as an internal reference ($n=3$ replicates). J, K: Immunoblots (left) and relative protein expression levels (right) of TET3, DNMT3B, and KDM6B in placental tissues. α -Tubulin was used as an internal reference. ($n=3$ independent biological replicates). L: Detection of the expression levels of Tet3, Dnmt3b, Kdm4b, Kdm5b, and Kdm6b in placental tissues by RT-qPCR ($n=3$ independent biological replicates). Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.

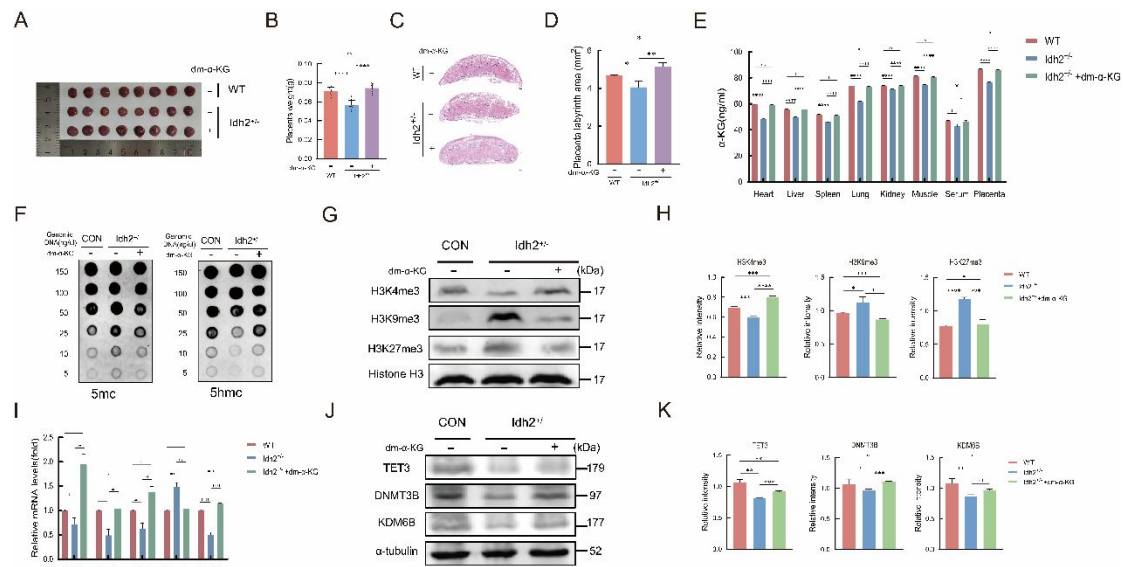


Figure 7 α -KG rescues placental developmental defects in $Idh2^{+/-}$ *M. musculus* by restoring DNA and histone methylation modification levels

A, B: Placental morphology (A) and statistical analysis of placental weights (B) of WT, $Idh2^{+/-}$, and $Idh2^{+/-}$ + dm- α -KG *M. musculus*. Placental weight were calculated as the mean value per litter, and data represent mean $\pm$ SEM. The data are from 10 litters of independently pregnant *M. musculus* ($n=10$ placentas per group). C, D: HE-stained sections of placental tissues and quantitative analysis of the area of the labyrinth layer of WT, $Idh2^{+/-}$, and $Idh2^{+/-}$ + dm- α -KG placentas ($n=3$ independent biological replicates). E: Detection of α -KG content in different tissues and serum of pregnant WT, $Idh2^{+/-}$, and $Idh2^{+/-}$ + dm- α -KG *M. musculus* ($n=3$). F: Dot blot analysis of 5mc and 5hmc in placental tissues of WT, $Idh2^{+/-}$, and $Idh2^{+/-}$ + dm- α -KG *M. musculus*, respectively ($n=3$ independent biological replicates). G, H: Immunoblots and relative protein levels of H3K4me3, H3K9me3, and H3K27me3 in placental tissues of WT, $Idh2^{+/-}$, and $Idh2^{+/-}$ + dm- α -KG *M. musculus*. Histone H3 was used as an internal reference ($n=3$ independent biological replicates). I: RT-qPCR detection of the

expression levels of Tet3, Dnmt3b, Kdm4b, Kdm5b, and Kdm6b in placental tissues of WT, Idh2^{+/-}, and Idh2^{+/-} + dm-α-KG *M. musculus* (*n*=3 independent biological replicates). J, K: Immunoblots and relative protein levels of TET3, DNMT3B, and KDM6B in placental tissues of WT, Idh2^{+/-}, and Idh2^{+/-} + dm-α-KG *M. musculus*. α-Tubulin was used as an internal reference (*n*=3 independent biological replicates). Different significance levels are indicated by asterisks (*: *P*<0.05; **: *P*<0.01; ***: *P*<0.001; ****: *P*<0.0001) and ns: Not significant.

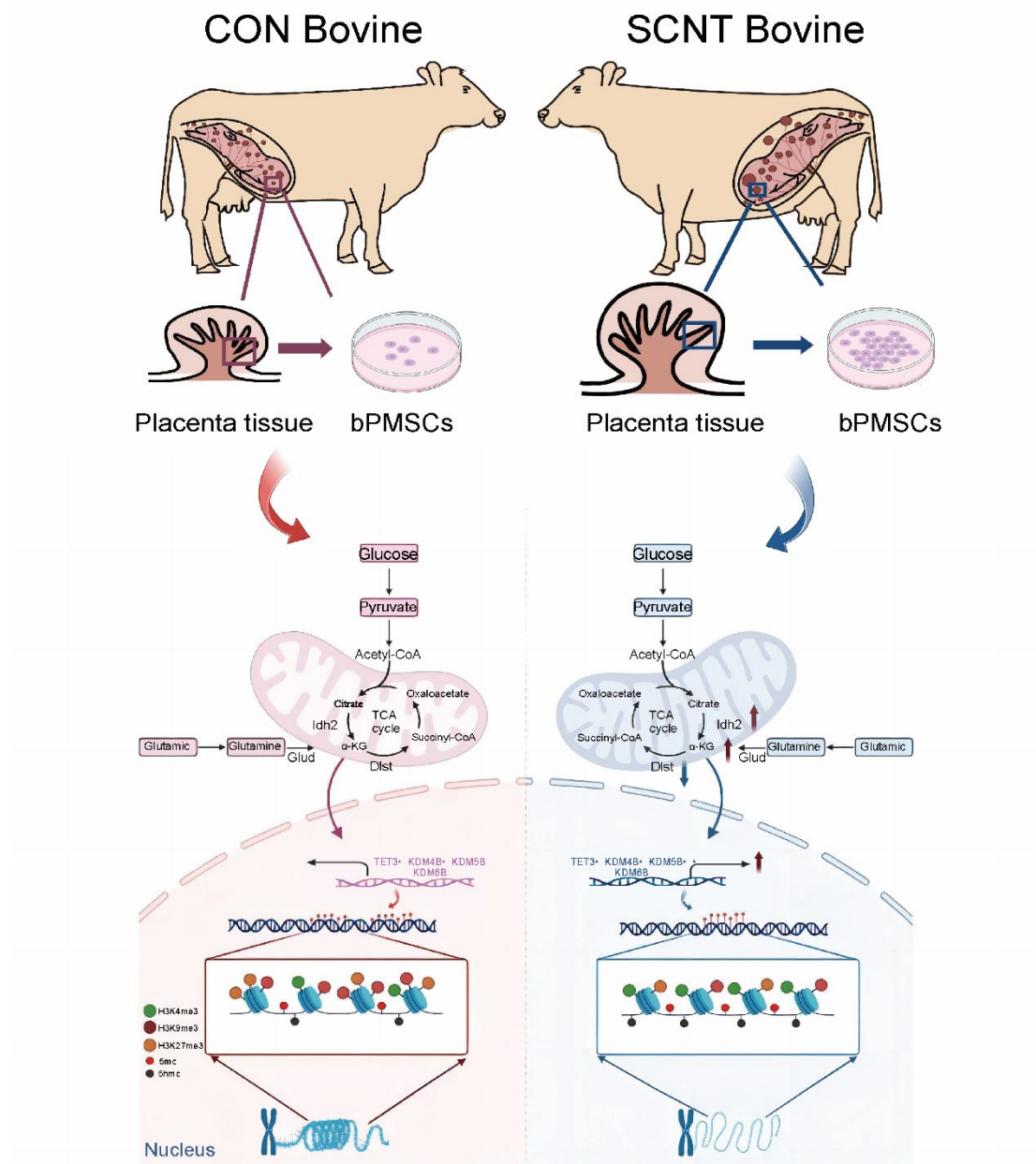


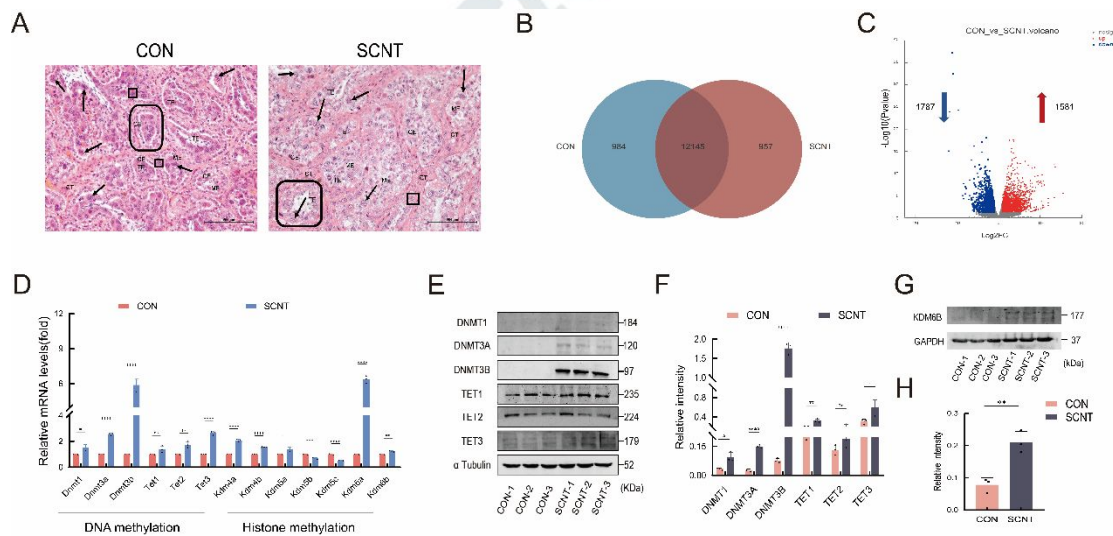
Figure 8 Schematic model of the Idh2- α -KG regulatory axis in SCNT *B. taurus*

placental hypertrophy

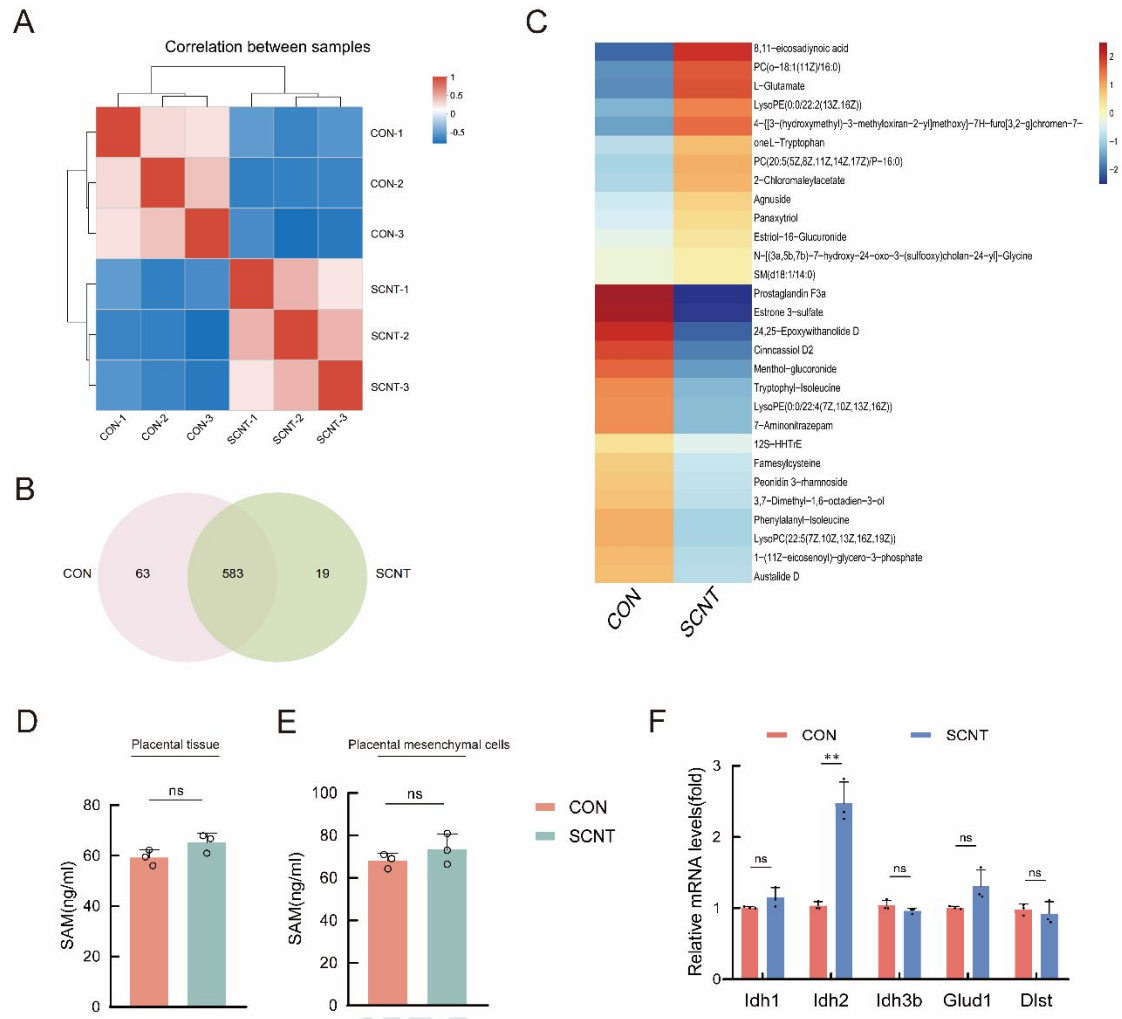
In SCNT *B. taurus* placentas and derived bPMSCs, Idh2 expression is significantly upregulated, enhancing the conversion of isocitrate to α -KG. The elevated α -KG levels

further activate TET3 and multiple lysine demethylases (KDM4B, KDM5B, KDM6B), leading to increased levels of 5hmC and H3K4me3, as well as decreased levels of H3K9me3 and H3K27me3. These epigenetic alterations affect the chromatin accessibility of key imprinted genes (e.g., upregulation of *Igf2r* and *Znf597*; downregulation of *Phlda2* and *Peg10*), ultimately resulting in placental hypertrophy. In contrast, CON placentas maintain normal α -KG levels and a balanced epigenetic modification state. Red arrows indicate upregulation or increased modification levels, while blue arrows indicate downregulation or decreased modification levels.

SUPPLEMENTARY FIGURE LEGENDS



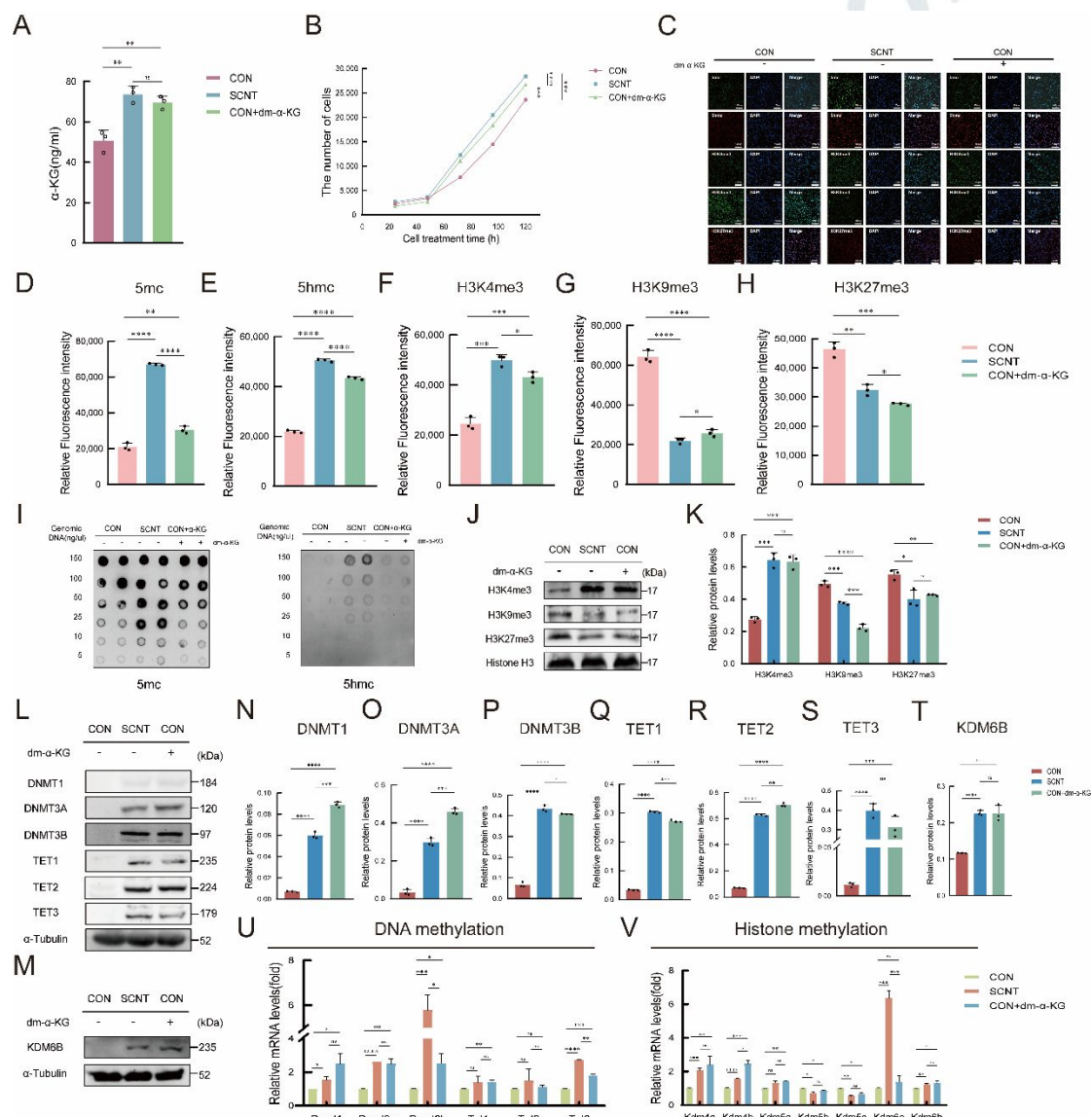
Mesenchyme; Arrows indicate trophoblast binucleate cells; Squares indicate crypt giant cells; Rounded squares indicate caruncular crypts. B: Screening of differentially expressed genes in placentomes from CON and SCNT *B. taurus* by RNA-seq. C: Volcano plot of up-and down-regulated differentially expressed genes in placentomes from CON and SCNT *B. taurus*. D: Detection of the expression of enzymes involved in DNA methylation and histone methylation in placental mesenchymal cells from CON and SCNT *B. taurus* by RT-qPCR ($n=3$ biologically independent replicates). E-H: Representative images and relative protein expression levels of DNMT1, DNMT3A, DNMT3B, TET1, TET2, TET3, and KDM6B in placental mesenchymal cells from CON and SCNT cows (*B. taurus*), respectively ($n=3$ biologically independent replicates). Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.



Supplementary Figure S2 The placentas of SCNT *B. taurus* exhibit a high level of α -KG, and the synthetase Idh2 is highly expressed

A: Sample correlation analysis of LC-MS for placentomes from CON and SCNT *B. taurus* ($n=3$ biologically independent replicates). B: Differential metabolites in placentomes from CON and SCNT *B. taurus* detected by LC-MS under negative ion mode. A total of 583 differential metabolites were identified between the two groups. C: Cluster analysis of differential metabolites in placentomes from CON and SCNT *B. taurus* in negative ion mode. D: Levels of SAM in placentomes from CON and SCNT *B. taurus* ($n=3$ biologically independent replicates). E: Levels of SAM in placental

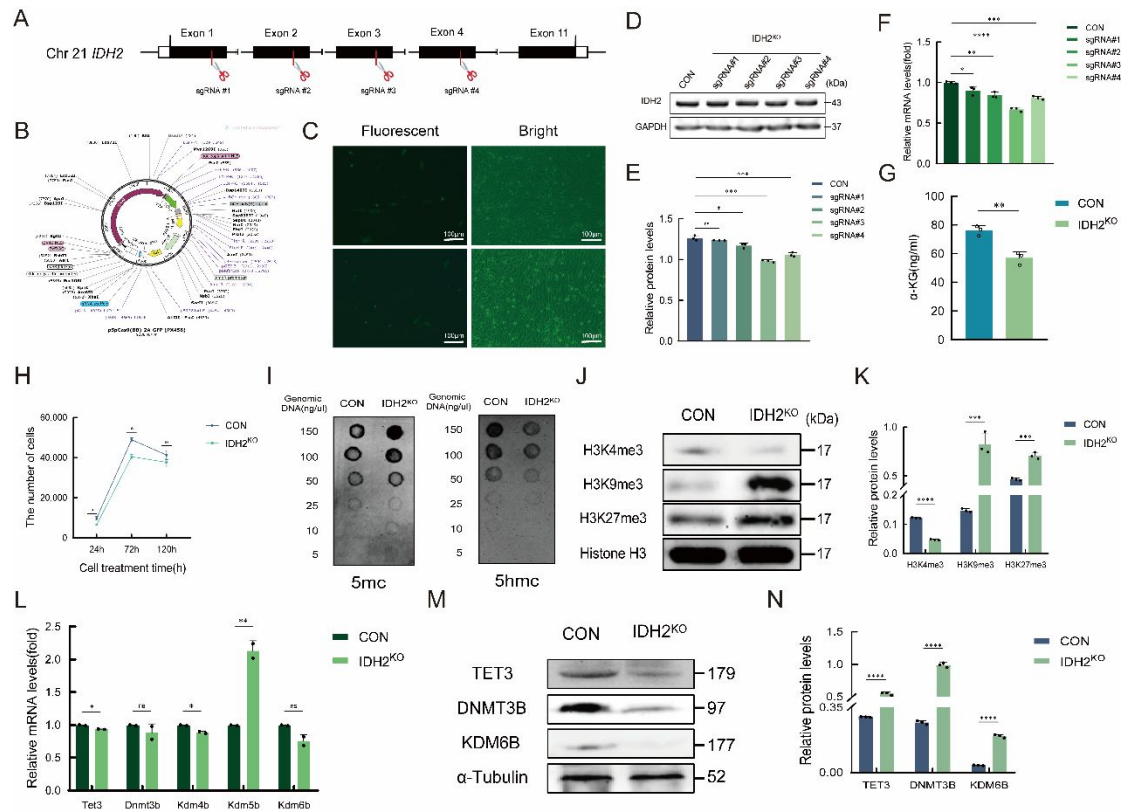
mesenchymal cells from CON and SCNT *B. taurus* ($n=3$ biologically independent replicates). F: Detection of the expression of enzymes involved in α -KG production in placental mesenchymal cells from CON and SCNT *B. taurus* by RT-qPCR ($n=3$ biologically independent replicates). Different significance levels are indicated by asterisks (**: $P<0.01$) and ns: Not significant.



Supplementary Figure S3 The Idh2- α -KG axis regulates epigenetic reprogramming in the placentas of CON *B. taurus*

1453 A: Detection of intracellular α -KG levels in placental mesenchymal cells from CON *B.*
 1454 *taurus* after α -KG supplementation by ELISA ($n=3$ biologically independent replicates).
 1455 B: Detection of cell proliferation ability after α -KG supplementation by CCK-8 assay.
 1456 C-H: Immunofluorescence staining and fluorescence intensity analysis of 5mc, 5hmc,
 1457 H3K4me3, H3K9me3, and H3K27me3 in placental mesenchymal cells from CON *B.*
 1458 *taurus* after α -KG supplementation. 5mc, H3K4me3, and H3K9me3 were stained green,
 1459 5hmc and H3K27me3 were stained magenta, and DAPI was stained blue ($n=3$
 1460 biologically independent replicates). The scale bar is 100 μ m. I: Representative images
 1461 of 5mc and 5hmc respectively ($n=3$ biologically independent replicates). J-K:
 1462 Representative images and relative protein levels of H3K4me3, H3K9me3, and
 1463 H3K27me3 in placental mesenchymal cells from CON *B. taurus* after α -KG
 1464 supplementation respectively. Histone H3 was used as a loading control ($n=3$
 1465 biologically independent replicates). L-T: Representative images and relative protein
 1466 expression levels of DNMT1, DNMT3A, DNMT3B, TET1, TET2, TET3, and KDM6B
 1467 in placental mesenchymal cells from CON *B. taurus* after α -KG supplementation
 1468 respectively. α -Tubulin was used as a loading control ($n=3$ biologically independent
 1469 replicates). U, V: Detection of the expression of DNA methylation and histone
 1470 methylation enzymes in placental mesenchymal cells from CON *B. taurus* by RT-qPCR
 1471 ($n=3$ biologically independent replicates). Different significance levels are indicated by
 1472 asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not
 1473 significant.

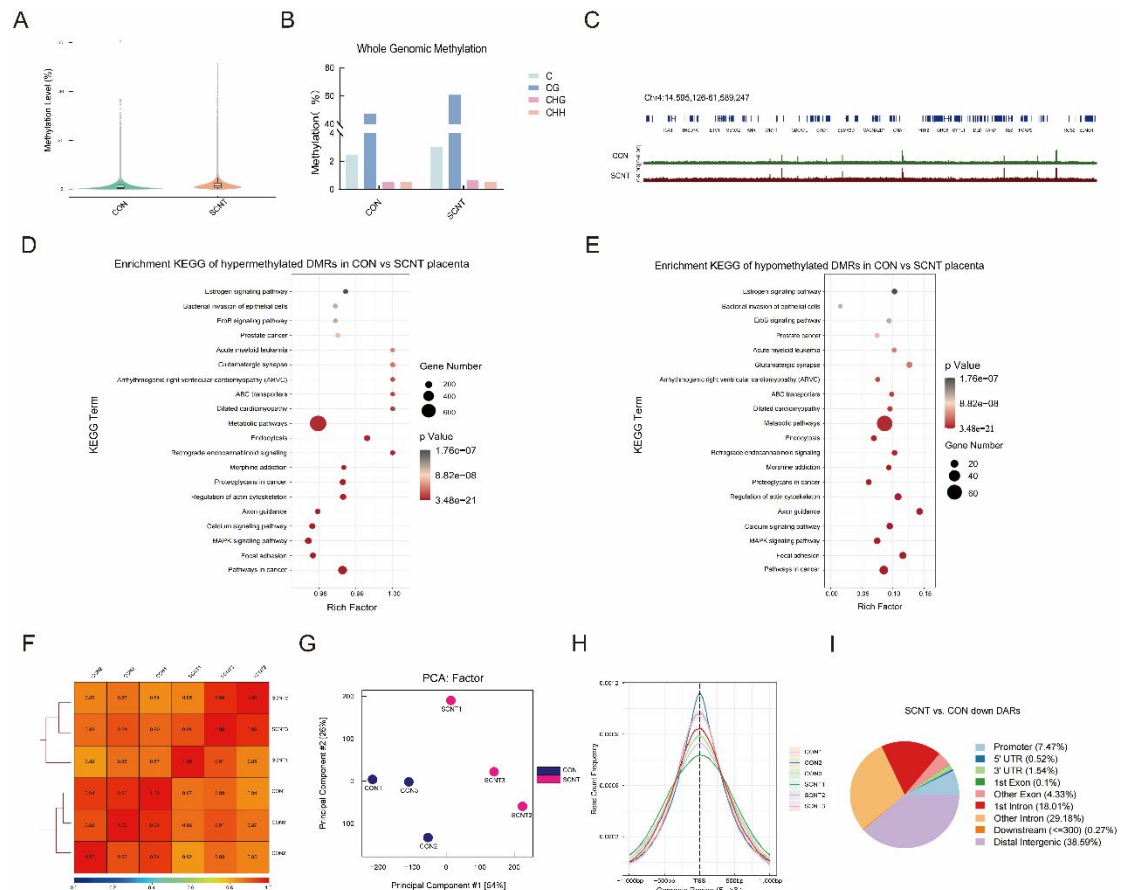
1474



Supplementary Figure S4 Knockdown of *Idh2* in cells affects the level of intracellular epigenetic modifications

A: Schematic diagram of the design of sgRNA for the *Idh2* gene. B: The backbone vector for knocking down *Idh2* using CRISPR-Cas9 technology. C: Fluorescence and bright-field images of cells transfected with the *Idh2*^{KO} vector. D, E: Schematic diagram and relative protein expression levels of *Idh2* in cells transfected with *Idh2* knockdown vectors constructed with different sgRNAs, respectively. GAPDH was used as a loading control ($n=3$ biologically independent replicates). F: Detection of the expression level of *Idh2* in cells transfected with *Idh2* knockdown vectors constructed with different sgRNAs by RT-qPCR ($n=3$ biologically independent replicates). G: Detection of the intracellular α -KG content after *Idh2* knockdown by ELISA ($n=3$ biologically independent replicates). H: Detection of cell proliferation ability after *Idh2*

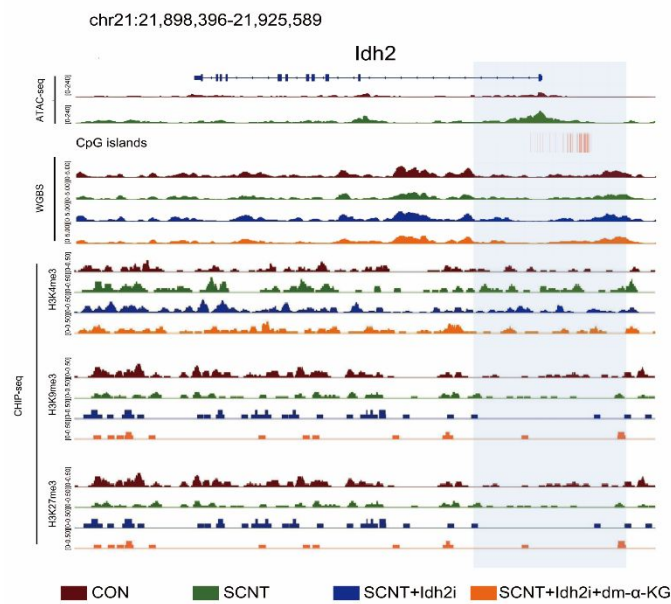
knockdown by CCK-8 assay. I: Representative images of 5mc and 5hmc in cells after Idh2 knockdown, respectively ($n=3$ biologically independent replicates). J, K: Representative images and relative protein levels of H3K4me3, H3K9me3, and H3K27me3 in cells after Idh2 knockdown, respectively. Histone H3 was used as a loading control ($n=3$ biologically independent replicates). L: Detection of the expression levels of Tet3, Dnmt3b, Kdm4b, Kdm5b, and Kdm6b in cells after Idh2 knockdown by RT-qPCR ($n=3$ biologically independent replicates). M, N: Representative images and relative protein levels of TET3, DNMT3B, and KDM6B in cells after Idh2 knockdown, respectively. α -Tubulin was used as a loading control ($n=3$ biologically independent replicates). Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.



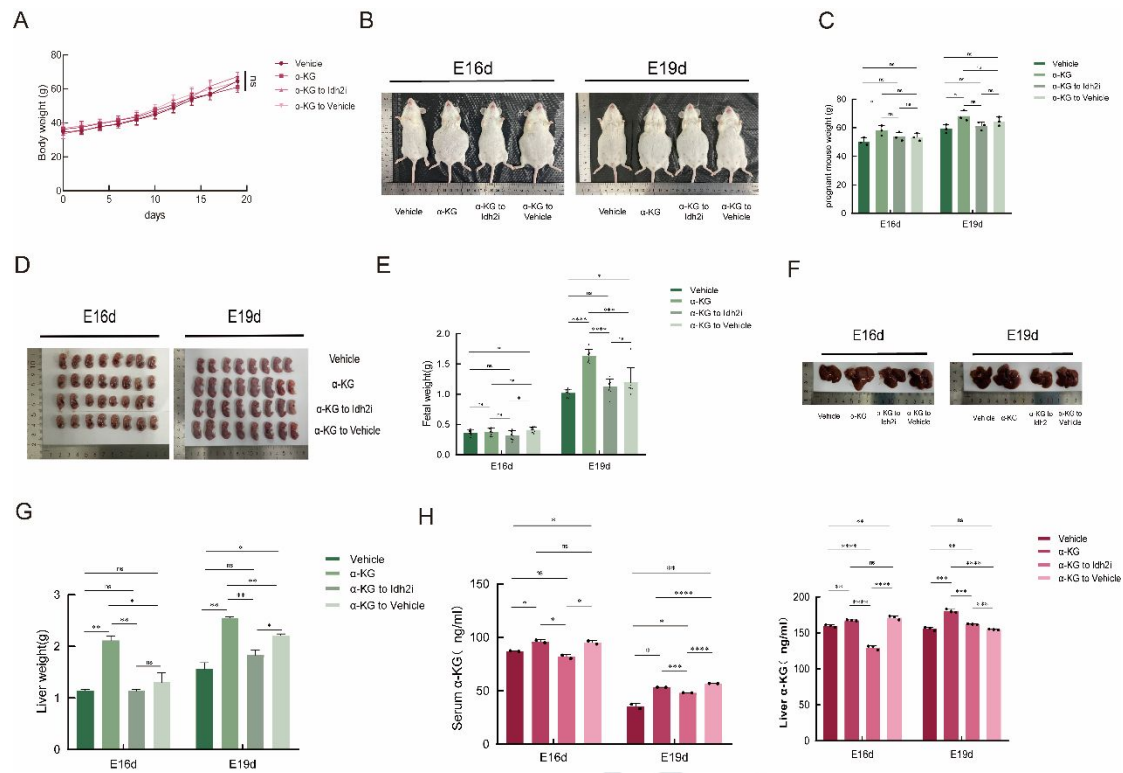
Supplementary Figure S5 Multi-omics reveals the effect of α -KG on epigenetic modifications in SCNT and the unique chromatin openness characteristics of the placentas of SCNT *B. taurus*

A: Violin plots of the genome-wide methylation levels in placentalomes from CON and SCNT *B. taurus*. B: Proportions of methylated C, CG, CHG, and CHH in the genome of placentalomes from CON and SCNT *B. taurus*. C: DNA methylation modification levels on chromosome 14 in placentalomes of CON and SCNT *B. taurus*. D: KEGG analysis of hypermethylated DMRs in placentalomes from CON and SCNT *B. taurus*. E: KEGG analysis of hypomethylated DMRs in placentalomes from CON and SCNT *B. taurus*. F: Sample correlation analysis of ATAC in placentalomes from CON and SCNT *B. taurus* ($n=3$ biologically independent replicates). G: PCA analysis of ATAC in

placentomes from CON and SCNT *B. taurus*. H: Visualization of ATAC sequencing signals covering the entire genome centered around the TSS ($n=3$ biologically independent replicates). I: Genomic distribution of down-regulated differentially accessible regions between placentomes from CON and SCNT *B. taurus*.



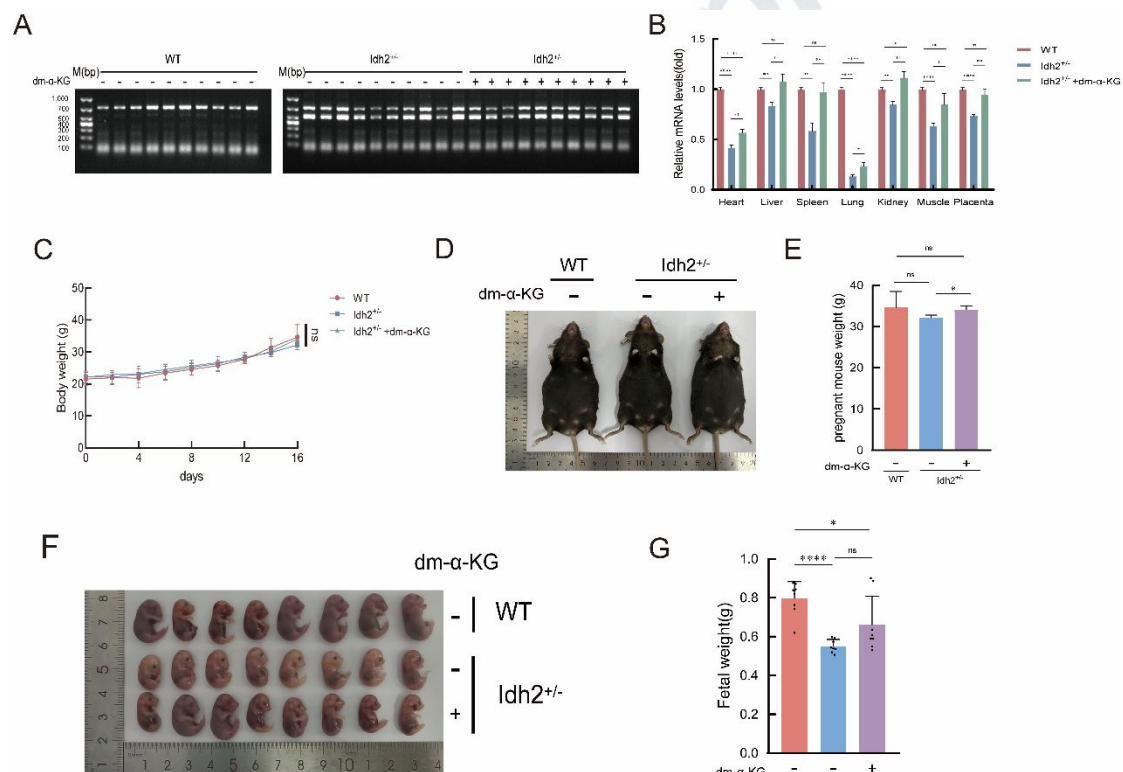
Supplementary Figure S6 Epigenetic map and chromatin openness of *Idh2*



Supplementary Figure S7 Supplementation of α -KG and drug intervention during pregnancy regulate abnormal placental hyperplasia through epigenetic modifications

A: Changes in the body weight of pregnant female mice after supplementation with α -KG and pharmacological intervention with an Idh2 inhibitor. Body weights are presented as mean $\pm$ SEM. B, C: Schematic diagrams and body weight statistics of female *M. musculus* at E16 and E19 after supplementation with α -KG and pharmacological intervention with an Idh2 inhibitor ($n=3$ biologically independent experiments). Data represent mean $\pm$ SEM. D, E: Pictures and weight statistics of fetuses at E16 and E19 after supplementation with α -KG and pharmacological intervention with an Idh2 inhibitor. Fetal weights were calculated as the mean value per litter, and data represent mean $\pm$ SEM. F, G: Schematic diagrams and weight statistics of the livers

of female *M. musculus* at E16 and E19 after supplementation with α -KG and pharmacological intervention with an Idh2 inhibitor. Data represent mean $\pm$ SEM. H: Detection of the α -KG content in the serum and liver of female mice at E16 and E19 after α -KG supplementation and Idh2 pharmacological interference by ELISA, respectively ($n=3$ biologically independent replicates). Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$) and ns: Not significant.



Supplementary Figure S8 α -KG rescues placental developmental defects in Idh2^{+/-}

***M. musculus* by restoring DNA and histone methylation modification levels**

A: Genotype identification diagrams of WT, Idh2^{+/-} and Idh2^{+/-} + dm- α -KG ($n=10$). B:

Detection of the expression of Idh2 in different tissues of pregnant *M. musculus* of WT,

Idh2^{+/-} and Idh2^{+/-} + dm- α -KG genotypes by RT-qPCR ($n=3$ biologically independent replicates). C: Body weights of WT, Idh2^{+/-} and Idh2^{+/-} + dm- α -KG female *M. musculus* during pregnancy. Data represent mean $\pm$ SEM. D, E: Pictures and body weight statistics of E16 pregnant mice of WT, Idh2^{+/-} and Idh2^{+/-} + dm- α -KG genotypes. Data represent mean $\pm$ SEM. F, G: Pictures and weight statistics of fetuses from WT, Idh2^{+/-} and Idh2^{+/-} + dm- α -KG *M. musculus*. Fetal weights were calculated as the mean value per litter, and data represent mean $\pm$ SEM. ($n=10$ litters). Different significance levels are indicated by asterisks (*: $P<0.05$; **: $P<0.01$; ****: $P<0.0001$) and ns: Not significant.

SUPPLEMENTARY TABLES

Supplementary Table S1 Antibodies used in this paper

Primary antibodies	Vendor	Dilution	Source
TET1 (WB)	Santa Cruz Biotechnology (SC-293186)	1:200	Mouse
TET2 (WB)	Santa Cruz Biotechnology (SC-398535)	1:200	Mouse
TET3 (WB)	Santa Cruz Biotechnology (SC-518126)	1:200	Mouse
DNMT1 (WB)	Santa Cruz Biotechnology (SC-271729)	1:200	Mouse
DNMT3A (WB)	Santa Cruz Biotechnology (SC-373905)	1:200	Mouse
DNMT3B (WB)	Cell Signaling Technology (57868)	1:1000	Mouse
KDM6B (WB)	Proteintech (61387)	1:1000	Mouse
5mc (Dot blot/IF)	Cell Signaling Technology (12640)	1:1000	Rabbit
5hmc (Dot blot/IF)	Cell Signaling Technology (97763)	1:1000	Mouse
H3K4me3 (WB/IF/CHIP)	Abcam (ab8580)	1:1000	Rabbit
H3K9me3 (WB/IF/CHIP)	Abcam (ab8898)	1:1000	Rabbit
H3K27me3 (WB/IF/CHIP)	Abcam (ab6002)	1:1000	Mouse
GAPDH (WB)	Proteintech (60004-1-Ig)	1:5000	Mouse
α -Tubulin (WB)	Proteintech (11224-1-ap)	1:5000	Rabbit
Histone H3 (WB)	Proteintech (17168-AP)	1:1000	Rabbit

Secondary antibodies

HRP-conjugated affinipure goat anti-mouse IgG (WB/Dot blot)	Proteintech (SA00001-1)	1:5000	Goat
HRP-conjugated affinipure goat anti-rabbit IgG (WB/Dot blot)	Proteintech (SA00001-2)	1:5000	Goat
CoraLite488- conjugate goat anti- mouse IgG (IF)	Proteintech (SA00013-1)	1:1000	Goat
CoraLite488- conjugate goat anti- rabbit IgG (IF)	Proteintech (SA00013-2)	1:1000	Goat
CoraLite594- conjugate goat anti- mouse IgG (IF)	Proteintech (SA00013-3)	1:1000	Goat
CoraLite594- conjugate goat anti- rabbit IgG (IF)	Proteintech (SA00013-4)	1:1000	Goat

1562

1563

Supplementary Table S2 List of sgRNA specific primers used this study

Exon	Sense	antisense
1	caccAGAGCGAGCGTACGACTC GC	aaacGCGAGTCGTACGCTCGCTC T
2	caccCGCCACCTTGATCCTCTTG T	aaacACAAGAGGATCAAGGTGG CG
3	caccGGTCACCATCGACTCCGC GT	aaacACGCGGAGTCGATGGTGA CC
4	caccAACATCCCGCGTCTCGTC CC	aaacGGGACGAGACGCGGGATG TT

Supplementary Table S3 List of RT-qPCR specific primers used this study

Genes	Genebank	Forward primer sequence	Reverse primer sequence
<i>B-Gapdh</i>	NC_037332.1	GATGGTGAAGGTCG GAGTGAAC	GTCATTGATGGCGACGA TGT
<i>B-Tet1</i>	NC_037355.1	CAAAACCAGGTGGC GCTTG	GCAGGCTCTGTTTTATTT CCAC
<i>B-Tet2</i>	NC_037333.1	AACCAAGCAAATGC GTTCCC	TGGATCCAGGCTCGACCT TA
<i>B-Tet3</i>	NC_037338.1	CCTACGGTGGTGCT GAGTTT	ATACTCCTTGGGGCCTGG AT
<i>B-Dnmt1</i>	NC_037334.1	GGCCCAATGAGACA GACATCAA	TCCTCATCGCTCCAGTAA AGCA
<i>B-Dnmt3a</i>	NC_037338.1	CTCCGAAGGTTTAC CCACCTG	GATGTAGCGGTCCACCT GAATG
<i>B-Dnmt3b</i>	NC_037340.1	CCATGGTGGTGTCC TGGAAG	GCCAAGTTAAAGTGCTG GCTGA
<i>B-Kdm4a</i>	NC_037330.1	CAGCGATGATGAGC TACCAGA	CTCCAAACTCCACCTGAT GG
<i>B-Kdm4b</i>	NC_037334.1	TGCCGGAAACGGAT GAAGAA	TAGTAAAGCCCGTTGCG GTT
<i>B-Kdm5a</i>	NC_037332.1	CGCCATTGTGTCTT CTCA	CACTGTCGCTCATCATCA
<i>B-Kdm5b</i>	NC_037343.1	CTTCGCCTTCATCC ACAA	TCAGAATCCGCTCGTAGT
<i>B-kdm5c</i>	NC_037357.1	AGTGGTGAAGGAG GAGTC	GAGGCAACAAGCAGAAG A
<i>B-Kdm6a</i>	NC_037357.1	ACAAAACCTGGCAAC ATAATACAG	AAAGTTGACCAAATAAA GACTTA
<i>B-Kdm6b</i>	NC_037346.1	CTCTTTTCTTTTAA GCGTGAAACAG	CAAAAAACAACCGACAA AACGA
<i>B-Idh1</i>	NC_037329.1	GGGCTTCATTTGGG CCTGTA	CTACTGTCTTGCCATCTG GACAAAC
<i>B-Idh2</i>	NC_037348.1	CTGGCACATTCAAG GTGGTC	CTGGAAGCAGCTGTGTG CAA
<i>B-Idh3b</i>	NC_037340.1	GCAGGTGCTGAGTT CCATGAAG	ATGGACCACATTGGCAA ACA
<i>B-DLud1</i>	NC_037355.1	TGTACCCACAGCAG AGTTCCAAG	TTCTCGATGGCGTTGACG TAG
<i>B-Dlst</i>	NC_037337.1	CAGCCTTTGCCTTG CAGGA	ACCGCAACGCTGATGTC AATATAA
<i>B-Znf597</i>	NC_037352.1	TAAGAACCAGAGCA CTTCC	CCACAGTCATCACATTCA C

<i>B-Peg10</i>	NC_03 7331.1	GTGAACTTCAGGCA GGCAGAATA	TGGATCAATTCAGGAGT CCACA
<i>B-Phlda2</i>	NC_03 7356.1	CGGTGCTACTCTAC TTCACACACA	ACAGATGGTTTGGATCA ACAGG
<i>B-Igf2r</i>	NC_03 7336.1	GTCCTCCACCATCT TCTTC	CCTCCTCCTCCTTGTTCA
<i>B-Snrpn</i>	NC_03 7348.1	CGTATATTCATTGG CACCTT	GTCATTACCTGTTGAGAT GG
<i>B-Magel2</i>	NC_03 7348.1	TGCCTCTTCTAATG CCTTC	CACTACTATCCTCCTCTT CTG
<i>M-Gapdh</i>	NC_00 0072.7	TCTCCTGCGACTTC AACA	TGTAGCCGTATTCATTGT CA
<i>M-Idh2</i>	NC_00 0073.7	GACAAGCACTATAA GACTGAC	TCTGGTGTTCTCGGTAAT G
<i>M-Dnmt1</i>	NC_00 0075.7	CTGTTCTCCTTCTA CCATTA	ATTCTCCTTGTCCTCCTT ATC
<i>M-Dnmt3a</i>	NC_00 0078.7	CCAGAAGAAGAGA AGAATCCT	GGTAAGCACACTCCAAG AA
<i>M-Dnmt3b</i>	NC_00 0068.8	ATGACTCTGCTGCT TCTG	CCTTGTTGTTGGTGACTT C
<i>M-Tet1</i>	NC_00 0076.7	GACCTGCCTATCTT CCTTC	CTTCCTCCTCTCCACCAT
<i>M-Tet2</i>	NC_00 0069.7	GCCAGGAGAAGAA GATACG	ACAATGAGAACCAACAG ACT
<i>M-Tet3</i>	NC_00 0072.7	GCTGTCTCACTCCTT CAC	TTCCTCCTCGTCCTCTTC
<i>M-Kdm4a</i>	NC_00 0070.7	CGCTACATTGCCTA CATTG	GAACTCACGAACGGTCA T
<i>M-Kdm4b</i>	NC_00 0083.7	CACTAACTTCGCCA CACT	CTTGCTTCCATTGCTCAT AG
<i>M-Kdm5a</i>	NC_00 0072.7	AAGATGTTGCCAGA AGAAGA	TCACCTCCTCCAGTTGTT
<i>M-Kdm5b</i>	NC_00 0067.7	GTTAGTCGCAGAAG AAGGT	TGTCGGAGGTCAGGTTA G
<i>M-Kdm5c</i>	NC_00 0086.8	GTTCTTGCTACGC TCTC	AGTTCCTCCTTGCTTCT A
<i>M-Kdm6a</i>	NC_00 0086.8	CAGGAAGTGGAAGT AATGGA	GAGTGGAGTTAGATAGT TGGT
<i>M-Kdm6b</i>	NC_00 0077.7	TGCTCAGTCAACAT CAACA	CACCAGGAACCAGTCAA G

1570

1571



Supplementary Table S4 Number of characteristic cells in CON and SCNT bovine placenta ($\bar{x} \pm SD$)

Analysis category	CON	SCNT
The number of trophoblast binucleate cells	18.4 $\pm$ 1.85	23.67 $\pm$ 1.25**
The number of trophoblast mononuclear cells	301 $\pm$ 6.34	242.33 $\pm$ 3.68***
The number of crypt giant cells	7 $\pm$ 1.41	13.4 $\pm$ 1.85***
Crypt area (μm^2)	7628.36 $\pm$ 307.68	13296 $\pm$ 808.75***

Note: **P<0.01, ***P<0.001.

IDH2- α -酮戊二酸通路紊乱扰乱表观遗传修饰与印记基因表达，诱发体细胞核移植牛胎盘肥大

赵晓雨^{1,2,#}, 云元^{1,2,#}, 廖鸿艺^{1,2}, 陶绍英^{1,2}, 吴珊珊^{1,2}, 高雨馨^{1,2}, 薄纯婕^{1,2}, 杨磊^{1,2}, 刘雪霏^{1,2}, 宋丽爽^{1,2}, 白春玲^{1,2}, 李光鹏^{1,2,*}, 苏广华^{1,2,*}

¹ 内蒙古大学 草原家畜生殖调控与繁育国家重点实验室, 内蒙古呼和浩特 010070, 中国

² 内蒙古大学 生命科学学院, 内蒙古呼和浩特 010070, 中国

*通讯作者, 电子邮箱: gpengli@imu.edu.cn; guanghuasu@imu.edu.cn

摘要

胎盘肥大是导致体细胞核移植 (SCNT) 动物妊娠失败的核心诱因, 代谢-表观遗传交互调控被认为是关键调控靶点, 但该过程的分子调控机制尚不明确。异柠檬酸脱氢酶 2 (IDH2) 是线粒体的关键代谢酶, 负责催化生成 α -酮戊二酸 (α -KG), 而 α -KG 是调控表观修饰的核心辅因子。本研究以体细胞核移植牛 (*Bos taurus*) 胎盘子叶及其分离的胎盘间充质细胞为试验材料, 发现 SCNT 样本中 α -KG 含量异常升高。细胞功能试验证实, α -KG 积累会造成 DNA 羟甲基化 (5hmC) 与活化型组蛋白修饰 H3K4me3 水平显著上调, 抑制型修饰 H3K9me3、H3K27me3 水平下降; 同时伴随 TET 双加氧酶、赖氨酸去甲基化酶 (KDMs) 家族基因表达紊乱, 最终引发胎盘发育关键印记基因异常 (*Igf2r*、*Znf597* 表达上调, *Phlda2*、*Peg10*、*Magel2* 表达下调)。机制探究结果显示, α -KG 合成关键酶 IDH2 在 SCNT 牛胎盘内表达显著升高, 其启动子区发生低甲基化修饰、H3K4me3 富集增多, 染色质开放程度显著上升; 利用 CRISPR-Cas9 敲低 *Idh2* 基因或添加特异性抑制剂阻断 α -KG 合成, 可逆转上述表观遗传紊乱、缓解胎盘肥大表型。小鼠 (*Mus*

1600 *musculus*) 体内验证试验表明, 妊娠期母体补充 α -KG 可诱导子代胎盘肥大, 而
1601 抑制 IDH2 表达能够完全挽救该病理表型。本研究首次阐明 IDH2- α -KG 通路通
1602 过代谢-表观遗传轴调控胎盘发育的分子机制, 为提升体细胞克隆效率提供新靶
1603 点, 也为临床胎盘相关疾病 (如巨大胎盘) 的干预提供候选方向。

1604 **关键词**

1605 体细胞核移植; IDH2- α -酮戊二酸通路; 胎盘肥大; 代谢-表观遗传调控; DNA/组
1606 蛋白甲基化; 印记基因

1607