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RESEARCH

Trps1 regulates mouse zygotic genome activation and preimplantation embryo development via the PDE4D/AKT/CREB signaling pathway

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Abstract Despite zygotic genome activation (ZGA) is crucial for early embryonic development, its regulatory mechanism is still unclear in mammals. In the present study, we demonstrate that TRPS1, a maternal factor, plays an essential role in mouse early embryogenesis by regulating the transition from 2-cell to 4-cell embryos during preimplantation development. The absence of *Trps1* could leads to impaired ZGA through AKT/CREB signaling pathway. Furthermore, our findings suggest that TRPS1 may modulate the transcription of *Pde4d* to influence AKT and CREB phosphorylation. Interestingly, compared to *Trps1* knockdown alone, co-injection of *Trps1* siRNA and *Pde4d* mRNA significantly enhances the development rate of 4-cell embryos. Collectively, these results indicate a negative involvement of *Trps1* in mouse preimplantation embryo development by targeting the PDE4D/AKT/CREB pathway to regulate ZGA.

Keywords Tricho-rhino-phalangeal syndrome 1 · Phosphodiesterase 4d · Zygotic genome activation · AKT/CREB pathway

Introduction

With the implementation of China's three-child policy, the population's willingness to give birth has gradually increased. Unfortunately, a series of adverse factors, such as environmental pollution,

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delayed childbearing age and increased life stress, have up-regulated the incidence of infertility in recent years (Hansen 2020). According to (Qiao et al. 2021) the major report of China Maternal and Child Health, the infertility rate may expect to reach 18.2% in 2023. Although assisted reproductive technology (ART) is currently an effective therapeutic tool for infertility in clinical practice, it also suffers from a low rate of implantation and a high rate of miscarriage (Audibert and Glass 2015). Zygotic genome activation (ZGA) failure, as a common cause of abnormal early embryonic development, is the main factor that leads to preimplantation embryo developmental arrest. Therefore, it is urgent to identify key transcription factors in the process of ZGA, which would contribute to a deeper understanding of ZGA and enhance the success rate of ART.

In mammals, the process by which sperm and egg unite to form fertilized eggs and subsequently develop into a blastocyst is referred to as preimplantation embryonic development, which undergoes a series of precise dynamic regulation (Cockburn and Rossant 2010). After fertilization, the substances stored in the eggs (such as proteins and mRNA) promote the initiation of ZGA. In mice, Minor ZGA occurs in the mid-to-late 1-cell embryo and early 2-cell embryo, while major ZGA takes place during the G2 phase of the 2-cell embryo (Xia and Xie 2020; Xu et al. 2021). During the process of ZGA, maternal mRNAs are gradually replaced by zygotic mRNAs, and the embryo rely on its self-expressed mRNAs to continue to regulate subsequent development and complete the maternal zygotic transition (MZT) (Lee et al. 2014; Sha et al. 2019; Vastenhouw et al. 2019). For example, the transcriptional burst of *MuERV-L* during ZGA has the potential to regulate the stability of pluripotent factors, affecting the transition from totipotency to pluripotency during embryo development (Honda et al. 2024; Suemoto et al. 2007). *Zscan4d*, a significant member of the *Zscan4* gene family, is highly expressed in the process of ZGA. It plays a critical role in the development from the 2-cell stage to the 4-cell stage, as a reduction in *Zscan4d* expression can impede this progression (Falco et al. 2007). However, the regulatory mechanism of ZGA in mammals is not yet fully understood and remains to be further explored.

Currently, it has reported that ZGA could be regulated by epigenetic modifications, cell cycle progression and transcription factors (Ibarra-Morales et al. 2021; Liu et al. 2022; Shen et al. 2022). DUX family were considered as a critical inducer for ZGA process, which functions as pioneer factor to facilitate the access for other transcription factors by opening chromatin surrounding the TSS of early ZGA genes. *Dux* deficiency trigger to ZGA genes activation defective (such as *Zscan4*) and early embryo development failure (De Iaco et al. 2017; Hendrickson et al. 2017). However, *Dux* is essential for enhancing rather than initiating ZGA (Guo et al. 2019). NR5A2 has also been reported as a pioneer factor involved in ZGA, which binds to distal cis-regulatory elements and enhances chromatin accessibility to initiate ZGA (Gassler et al. 2022). OBOX belongs to PRD-like homeobox domain transcription factor family, which recruits RNA polymerase II to the promoter and distal enhancer of ZGA genes. *Obox* mutants would lead to ZGA and chromatin accessibility transition defective (Ji et al. 2023; Sakamoto et al. 2024).

It has been reported that members of the GATA family play an important role in early embryonic development. GATA3 is selectively expressed in mouse trophoblast cells to ensure normal embryonic development of pre- and post-implantation embryos (Home et al. 2017). In pig embryos, GATA4 can promote the expression of OCT4 during early trophoblast differentiation, maintain the dynamic balance of OCT4-CDX2, and ensure the smooth development of early embryos (Bou et al. 2022).

The tricho-rhino-phalangeal syndrome 1 (TRPS1) protein is an atypical GATA family that contains two nuclear localization signals and six C2H2 zinc finger structures, as well as a single GATA-binding motif and an Ikaros zinc finger structure (Momeni et al. 2000). *Trps1* has been shown to be critical for developmental and biological processes, including fetal kidney development (Gai et al. 2010, 2009), cartilage differentiation (Piscopo et al. 2009; Wuelling et al. 2009), and cell cycle progression (Sun et al. 2021; Wu et al. 2014). Our previous studies (Liu et al. 2019) have found that *Trps1* could act as an important ZGA regulator to participate in mouse blastocyst formation by affecting ZGA genes expression (e.g. *Zscan4d* and *MuERV-L*), but its mechanism is still unknown. In order to explore *Trps1* targeted genes during preimplantation embryo development, CUT&Tag was used to screen for genes regulated by the TRPS1 at the 2-cell embryo stage. The results demonstrated that *Trps1* was related to PI3K/AKT pathway,

and the *Pde4d* was found in the enriched gene. Our early research has confirmed that AKT/CREB pathway is involved in ZGA process (Chen et al. 2016). Inhibition of AKT or CREB activation would repress the 2-cell embryo/4-cell embryo transition. Therefore, we have a suspicion that TRPS1 may target *Pde4d* to regulated AKT/CREB pathway, thereby affecting ZGA genes expression.

Pde4d, located on the long arm of mouse chromosome 13 (13qD2.1), contains 17 exon fragments. The gene product is PDE4D, which belongs to the phosphodiesterase (PDE) family. The principal function of the PDE family is to hydrolyze cyclic adenosine monophosphate (cAMP), which plays an important regulatory role in cellular signaling pathways (Carvalho et al. 2021; Maurice et al. 2014). In 2008, large-scale transcriptome sequencing revealed that *Pde4d* is an incompletely imprinted gene (Babak et al. 2008). PDE4D shows paternal imprinting in embryonic day 9.5, suggesting that it may be involved in the regulation of embryonic development. In addition, *Pde4d* knockout mice exhibited growth retardation, reduced viability and decreased female fertility (Jin et al. 1999). However, the expression and role of *Pde4d* in preimplantation embryo development remain unexplored. The molecular mechanism of TRPS1 regulating *Pde4d* also needs further exploration.

In this study, we investigated the function of *Trps1* in mouse preimplantation embryos by RNAi technology, and CUT&Tag technology was used to observe the specific mechanism by which TRPS1 affects ZGA. Our study indicates that TRPS1 serves as a transcription factor that interacts with the promoter region of the *Pde4d* gene, promoting AKT/CREB signaling pathway activation, thereby regulating ZGA and 2-/4-cell embryo transition to complete the MZT process. Here, we propose a new insight into the function of *Trps1* in mouse blastocyst formation. And *Trps1* might serve as a candidate gene in clinical screening for high-quality fertilized eggs.

Materials and methods

Experimental animals

Kunming (KM) mice (females, 6–8 w; males > 8 w) were obtained from SLRC Laboratory Animal Co, Ltd (Shanghai, China), and housed for 3–5 d after adaptation. Mice were provided with water ad libitum and housed in conventional open-top cages (with 5 adult mice per cage). The study adheres to the 3Rs (Replacement, Reduction, Refinement) principles to minimize animal suffering.

Main reagents

M2 medium, KSOM medium (Nanjing Aibei Biotechnology Co, Ltd.); DEPC (Sigma-Aldrich, USA); Pregnant horse serum gonadotropin (PMSG) (Ningbo No.2 Hormone Production Company); Human chorionic gonadotropin (hCG) (ProSpec, Israel); Immunostaining fixative, 4,6-diamidino-2-phenylindole (DAPI) (Beyotime, China); SC-79 (MCE, USA); Glycogen, Rapid RNA™ MicroPrepR1050 kit (Roche, Switzerland); SYBR®Premix ExTaqTM (Vazyme, China); Reverse transcription kit and dNTP mixture (Thermo Fisher Scientific, USA); Hyperactive Universal CUT&Tag Assay Kit for Illumina (Vazyme, China); SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) (Cell Signaling Technologies, USA).

Mouse oocyte and preimplantation embryo collection

Female mice were administered with 10 IU PMSG and euthanized 48 h later, and germinal vesicle (GV) oocytes were isolated from the ovaries. Inject 6 IU hCG 46–48 h after PMSG injection, and euthanize at 21 h after post-hCG injection (p-hCG) to isolate MII oocytes from the fallopian tubes. Mouse 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst embryo were collected at 24, 42, 54, 64, 72, and 90 h of p-hCG, following mating with male and female mice.

CUT&Tag assay

Chromatin analysis was performed utilizing CUT&Tag, adhering to the protocol provided by the kit (Vazyme, China). Briefly, 200 2-cell embryos per group were combined with ConA beads for 10 min. Then, the ConA beads-embryos complex was incubated overnight with anti-TRPS1 or normal lgG control at 4 °C, followed by hatching secondary antibodies for 1 h and subsequent incubation of pG-Tn5 transposase for 1 h. pG-Tn5 transposase identified the target proteins through specific antibody and finished DNA sequences cleavage. The DNA sequences were

tagmented with adapters and further sent to Beijing Nuohe Zhiyuan Technology Co., Ltd. for library construction and sequencing. The information of antibodies is presented in Supplemental Table 1. Bioinformatics analysis used fot CUT &Tag assay is provided in Supplemental file. CUT &Tag data were available on GEO database (GSE283924).

Microinjection

As previously reported (Liu et al. 2019), Mouse 1-cell embryos were collected at 18 h p-hCG and subjected to siRNA microinjection after a pre-incubation period of 3 h. The sequence of siRNA was listed in Supplemental Table 1.

Immunofluorescence staining

Initially, the embryos were removed the zona pellucida through Tyrode treatment. Subsequently, the embryos were treated with 4% paraformaldehyde, 0.5% Triton X-100, and 0.2% BSA in sequence. Following the step, the embryos were incubated overnight at 4 °C with the primary antibody. The following day, the embryos were incubated with the secondary antibody and DAPI. Once the DAPI staining was completed, the embryos were visualized using a confocal microscope (Leica TCS SP8, Germany). At least three independent experiments were performed for each group. The information of antibodies is presented in Supplemental Table 1.

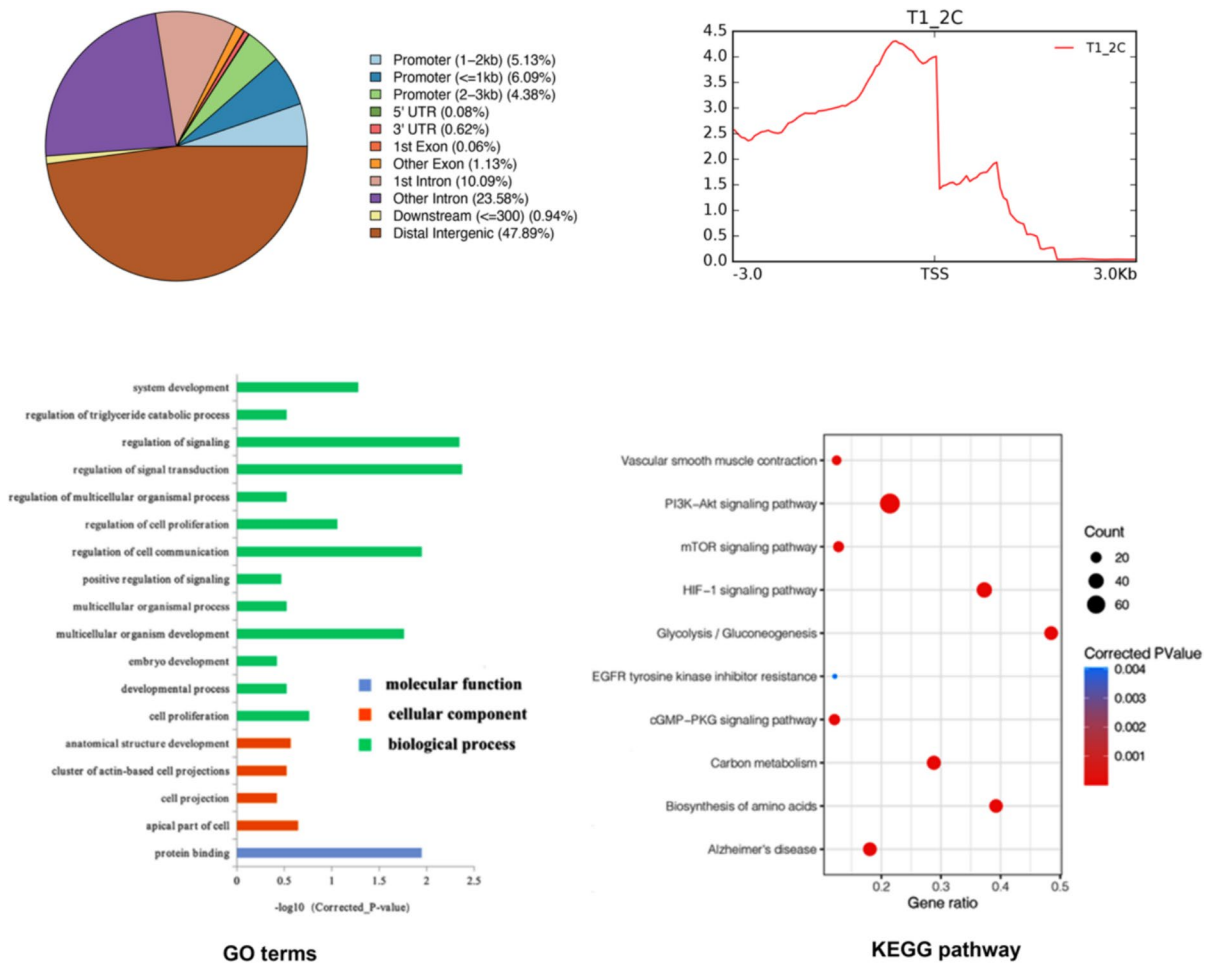


Fig. 1 TRPS1 CUT&Tag sequencing in 2-cell embryo. The gene corresponding to the TSS closest to the peak is considered a peak-related gene. GO terms for peak-related genes and KEGG pathway for peak-related genes

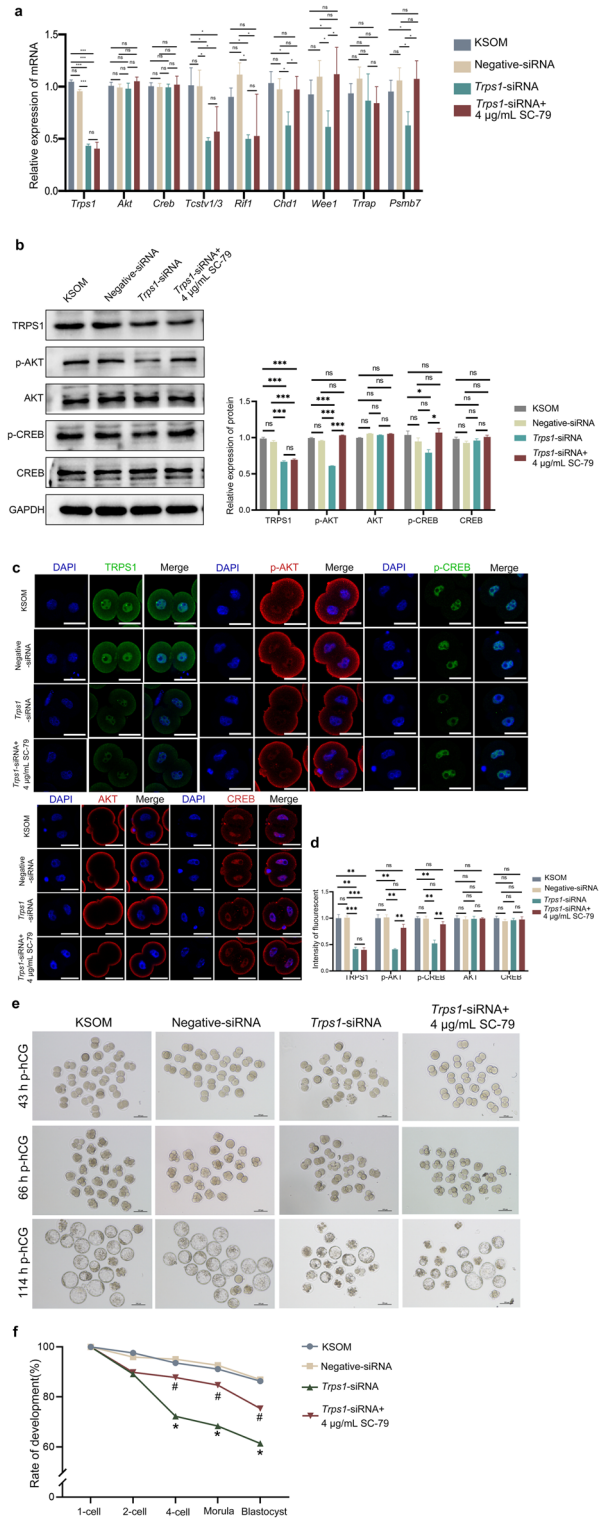
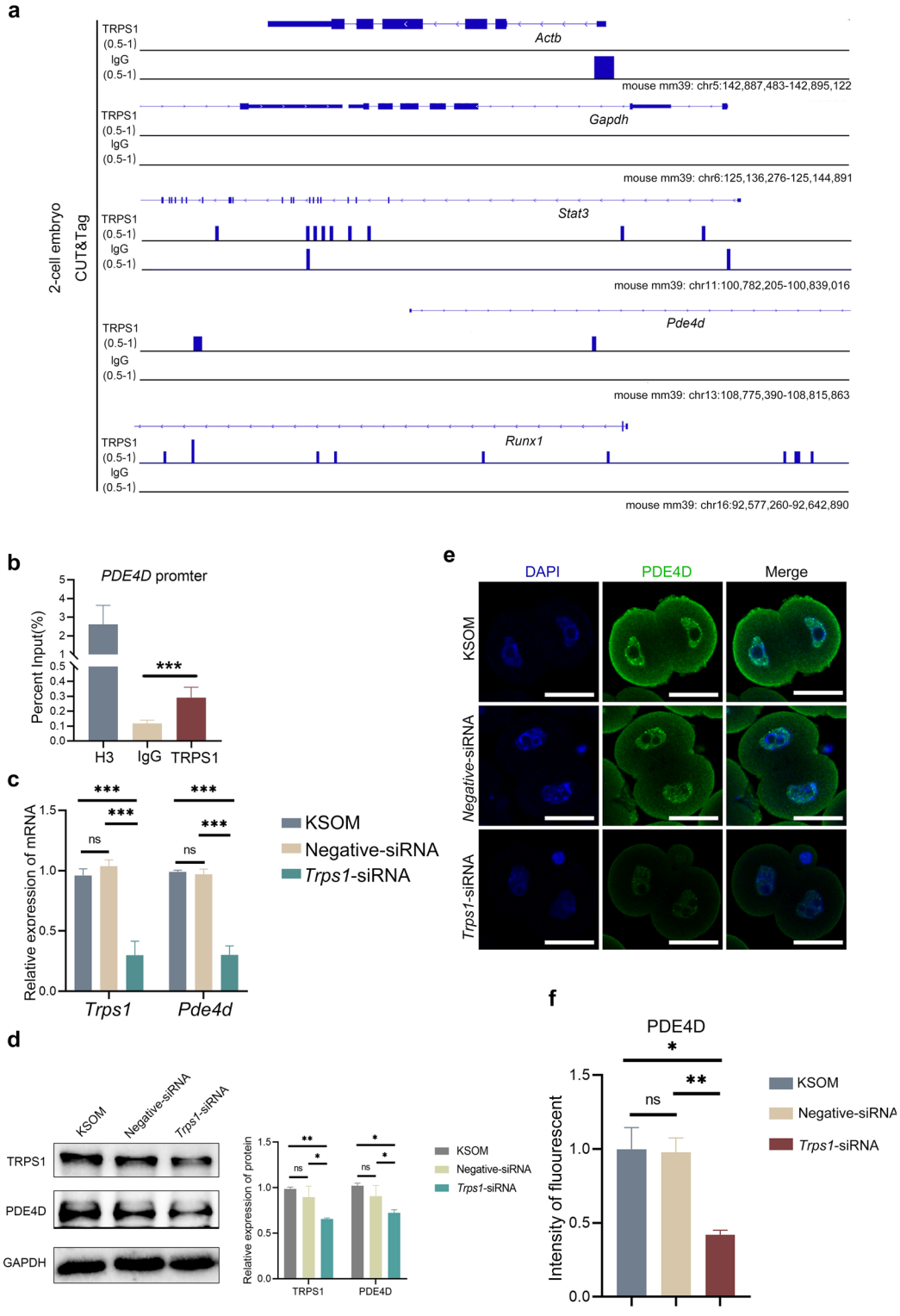


Fig. 2 AKT agonist SC-79 alleviate *Trps1* deficiency induced arrest of 2-cell embryo. **a**: RT-PCR analysis of *Akt*, *Creb* and ZGA marker genes upon SC-79 and *Trps1* siRNA treatment. One-way ANOVA: $^{***}P < 0.001$. Bars indicate standard deviation. **b-d**: Western blot (b) and Immunofluorescence staining (c, d) of AKT, p-AKT, CREB and p-CREB upon SC-79 and *Trps1* siRNA treatment. One-way ANOVA (Tukey correction): $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **e**: Sa: Representative images of mouse preimplantation embryos at different developmental stage after SC-79 and *Trps1* siRNA treatment. Scale bar, 100 μm. **f**: Line chart showed embryonic transition rate development. Chi-aquare($\chi 2$) test, *: Compared with KSOM group and Negative siRNA group, $P < 0.001$. $^{\#}$: Compared with *Trps1* siRNA group, $P < 0.001$. Scale bar, 50 μm. Bars in (a), (b) and (d) indicate SD for triplicate experiments


a
2-cell embryo CUT&Tag
TRPS1 (0.5-1)
IgG (0.5-1)
Actb
mouse mm39: chr5:142,887,483-142,895,122
Gapdh
mouse mm39: chr6:125,136,276-125,144,891
Stat3
mouse mm39: chr11:100,782,205-100,839,016
Pde4d
mouse mm39: chr13:108,775,390-108,815,863
Runx1
mouse mm39: chr16:92,577,260-92,642,890
b
PDE4D promter
Percent Input(%)
H3
IgG
TRPS1
c
Relative expression of mRNA
Trps1
Pde4d
KSOM
Negative-siRNA
Trps1-siRNA
d
KSOM
Negative-siRNA
Trps1-siRNA
TRPS1
PDE4D
GAPDH
Relative expression of protein
e
DAPI
PDE4D
Merge
f
PDE4D
Intensity of fluorescent

◂**Fig. 3** *Trps1* knockdown reduces Pde4d expression in mouse 2-cell embryo. **a**: CUT&Tag analysis on TRPS1 bound peaks present within or near the *Pde4d* gene. *Stat3* and *Runx1* as a positive control (Kanno et al. 2011; Suemoto et al. 2007). *Gapdh* and *Actb* as a negative control. **b**: TRPS1 ChIP-qPCR analysis in S-KO-V3 cell lines. **c**: RT-PCR analysis of *Pde4d* upon *Trps1* siRNA treatment. Student's t-test: $^{***}P<0.001$. Bars indicate standard deviation. **d-f**: Western blot (d) and Immunofluorescence staining (e. f) of PDE4D upon *Trps1* siRNA treatment. Student's t-test: $^{*}P<0.05$, $^{**}P<0.01$. Scale bar, 50 μm. Bars in (b), (c), (d) and (f) indicate SD for triplicate experiments

Real-time PCR

The Quick RNA Handbook™ MicroPrepR1050 kit was used for total RNA extraction as previously reported. A PCR amplifier (AB2720, Applied Biosystems, USA) was utilized for cDNA synthesis according to the reverse transcription kit manual. Amplification was conducted using SYBR®Premix ExTaqTM on a real-time PCR amplifier (PikoReal2.2.248; Thermo, USA). The sample is prepared in triplicate, with at least three independent replicates. The sequence information of the PCR primers used in this study is shown in Supplemental Table 2.

Western blot

120 embryos per group were added to the RIPA lysis buffer for sample preparation, and western blot was carried out as previously reported. The intensity of protein bands was calculated by ImageJ software. All primary antibodies used are showed in Supplemental Table 1.

Chromatin immunoprecipitation (ChIP)-qPCR

As our earlier reported, S-KO-V3 cells were collected and used for ChIP assays according to the protocol of the SimpleChIP Plus Enzymatic Chromatin Immunoprecipitation (IP) Kit. The purified DNA was subsequently analyzed through RT-qPCR using the following primers:

PDE4D-Pro-Forward:5′-CAGGTGAATGAGGCAGTGAG-3′,

PDE4D-Pro-Reverse: 5′-TAAATGCACGCTGCTAATGG-3′.

Statistical analysis

Developmental rates of embryos were calculated by the chi-square test. Real-time PCR data were assessed utilizing the $2^{-\Delta\Delta Ct}$ method to quantify relative mRNA expression levels. The student's test was administered utilizing SPSS 17.0 software, and histograms were generated using GraphPad Prism. The difference between the experimental and control groups ($P<0.05$) was considered statistically significant. Immunostaining experiments and protein blots were performed in at least three independent replicates with similar results.

Results

Targeted genes of TRPS1 in mouse 2-cell embryos

In order to investigate the function of *Trps1* in mouse preimplantation embryo, we conducted RNA-seq after *Trps1* knockdown in mouse 2-cell embryos. The results indicated that 1035 ZGA genes were differentially upregulated or downregulated following *Trps1* deficiency (Supplemental Fig. 1). In addition, CUT&Tag was used to screen for genes regulated by TRPS1 in mouse 2-cell embryo. 600 2-cell embryos were divided into Trps1 group and IgG control group. Gene Ontology of enriched genes between two groups revealed GO terms associated with "embryo development", "developmental process" and "system development" (Fig. 1a). The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis identified pathways that are associated with the PI3K/AKT signaling pathway (Fig. 1b). Moreover, 19 ZGA genes are directly regulated by TRPS1 and are associated with "cell differentiation" and "regulation of protein stability" (Supplemental Fig. 1).

Trps1 regulates phosphorylation levels of AKT and CREB in 2-cell embryos

Previous studies (Chen et al. 2016) have shown that the AKT pathway is closely related to ZGA, and activated AKT can phosphorylate a series of downstream substrates, such as CREB, affecting various cellular and physiological processes. To confirm the results of CUT&Tag analysis, we examined the expression of AKT and CREB after *Trps1* knockdown. Results indicated that the expression of *Akt* and *Creb* mRNA did not significantly change following *Trps1* deficiency (Fig. 2a). However, compared with controls, the phosphorylation of AKT and CREB were down-regulated in *Trps1* siRNA group (Fig. 2b-d). In addition, we further microinjected *Trps1* mRNA in *Trps1* deficiency embryo to observe the development

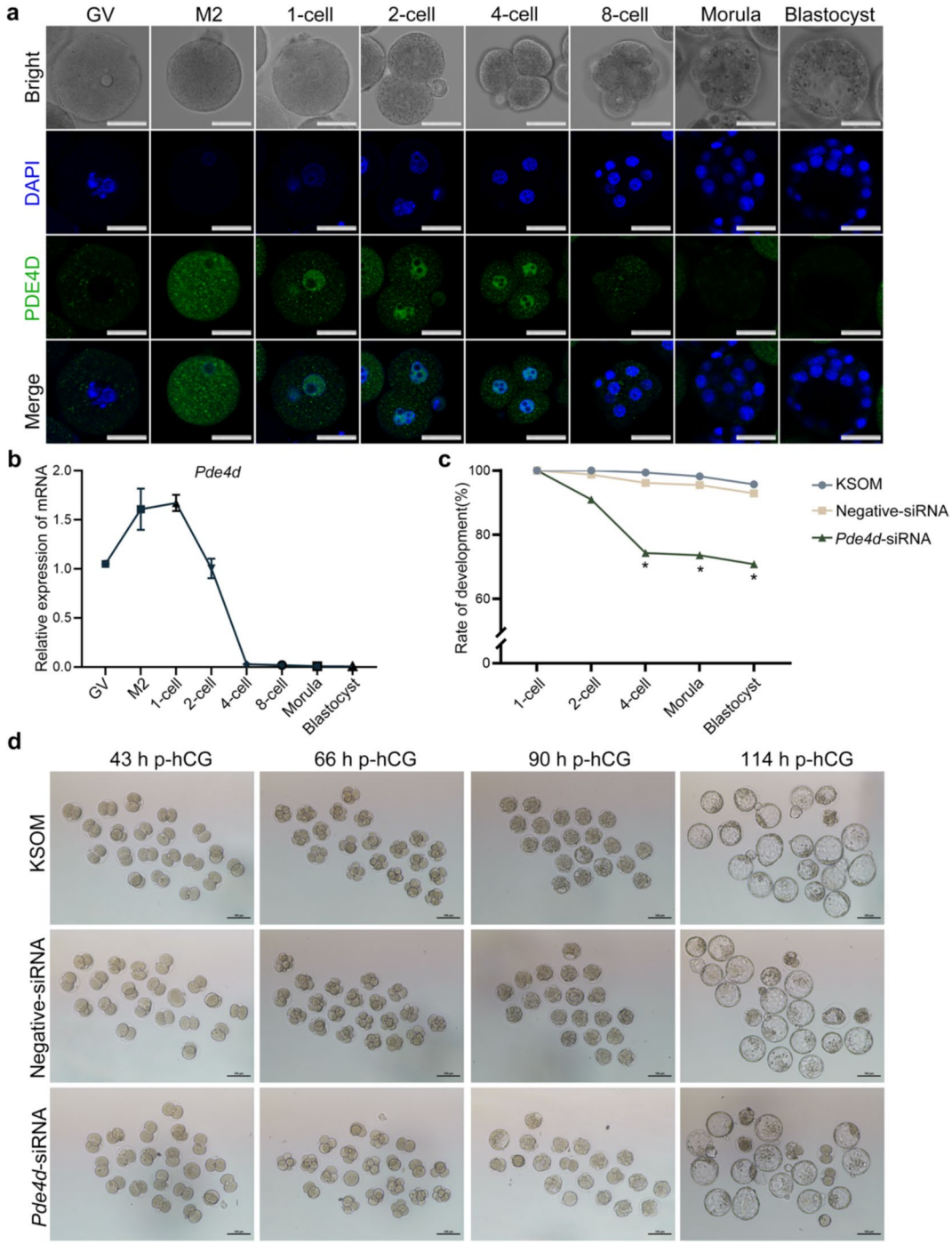


Fig. 4 The function of *Pde4d* in mouse preimplantation embryos. **a**: Immunofluorescence staining of PDE4D protein localization and expression in preimplantation embryos. Scale bar, 50 μm. **b**: Real-Time PCR of *Pde4d* mRNA in the preimplantation embryos. Bars indicate SD for triplicate experiments. **c**: Line chart showed embryonic transition rate development after *Pde4d* knockdown. Chi-aquare($\chi 2$) test, *: Compared with KSOM group and Negative siRNA group, $P < 0.001$. **d**. Representative images of mouse preimplantation embryos at different developmental stage after *Pde4d* knockdown. Scale bar, 100 μm

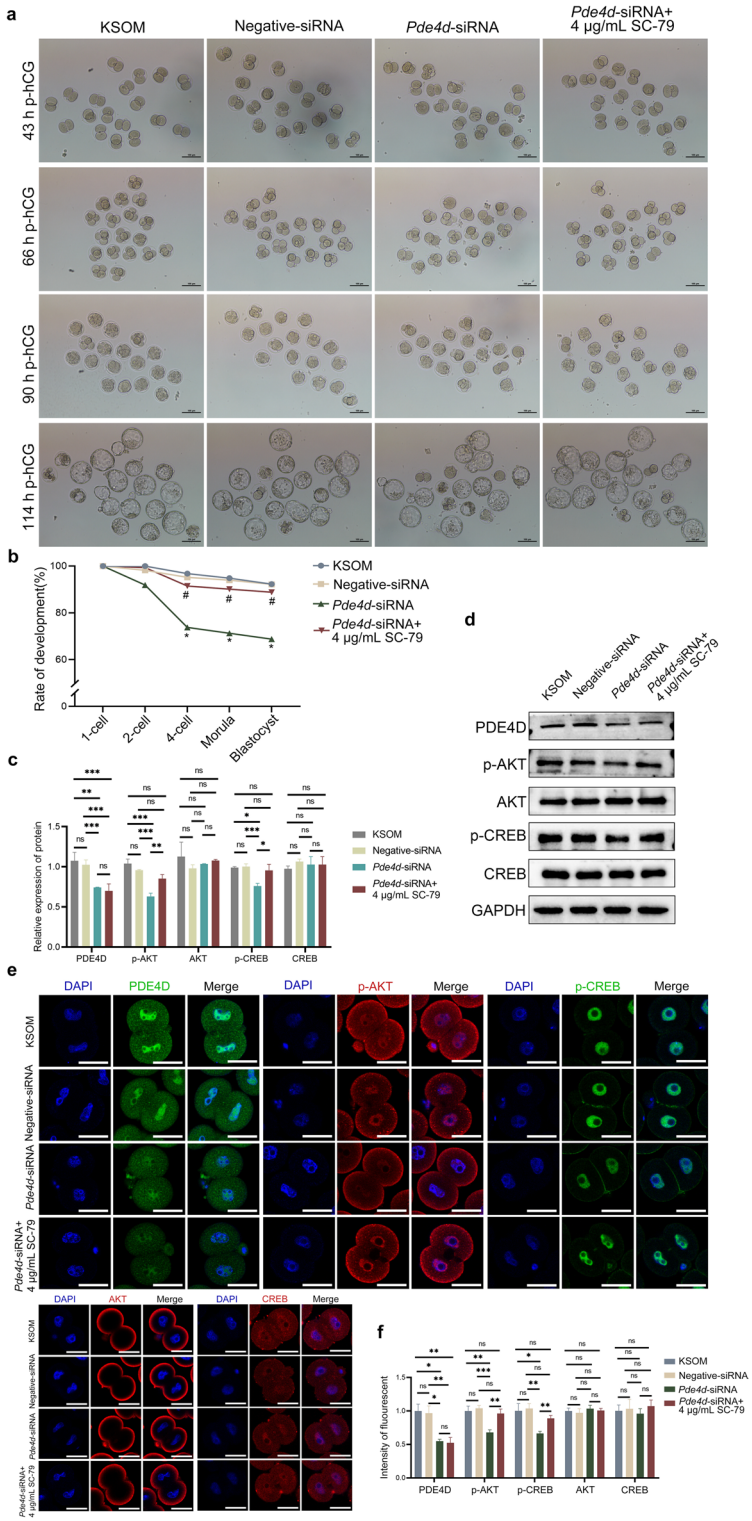


Fig. 5 AKT agonist SC-79 alleviate *Pde4d* deficiency induced arrest of 2-cell embryo. **a**: Representative images of mouse preimplantation embryos at different developmental stage after SC-79 and *Pde4d* siRNA treatment. Scale bar, 100 μm. **b**: Line chart showed embryonic transition rate development. Chi-aquare($\chi 2$) test, *: Compared with KSOM group and Negative siRNA group, $P < 0.001$. $^{\#}$: Compared with *Pde4d* siRNA group, $P < 0.001$. **c-f**: Western blot (c, d) and Immunofluorescence staining (e, f) of AKT, p-AKT, CREB and p-CREB upon SC-79 and *Pde4d* siRNA treatment. One-way ANOVA (Tukey correction): $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bar, 50 μm. Bars in (c) and (f) indicate SD for triplicate experiments

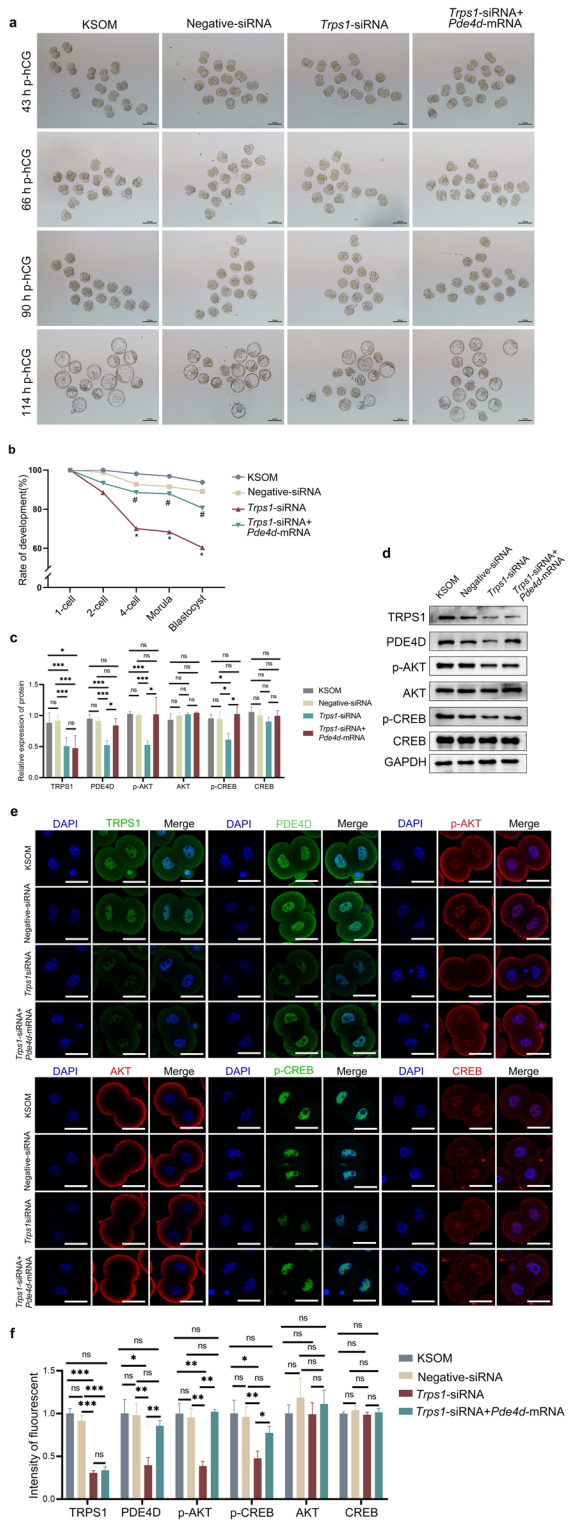


Fig. 6 Supplementing *Pde4d* alleviates *Trps1* deficiency ▶ induced arrest of 2-cell embryo. **a**: Representative images of mouse preimplantation embryos at different developmental stage after *Trps1* siRNA and *Pde4d* mRNA microinjection. Scale bar, 100 μm. **b**: Line chart showed embryonic transition rate development. Chi-aquare($\chi 2$) test, *: Compared with KSOM group and Negative siRNA group, $P<0.001$. $^{\#}$: Compared with *Trps1* siRNA group, $P<0.001$. **c-f**: Western blot (c, d) and Immunofluorescence staining (e, f) of AKT, p-AKT, CREB and p-CREB after *Trps1* siRNA and *Pde4d* mRNA microinjection. One-way ANOVA (Tukey correction): $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Scale bar, 50 μm. Bars in (c), and (f) indicate SD for triplicate experiments

of mouse preimplantation embryos. The results showed that supplementing *Trps1* could alleviate arrest of 2-cell embryos and the reduced activation of AKT and CREB caused by *Trps1* deficiency (Supplemental Fig. 2).

AKT agonist SC-79 alleviate *Trps1* deficiency induced arrest of 2-cell embryos

To further investigate whether *Trps1* participates in the regulation of the ZGA process via AKT/CREB pathway, 1-cell embryo were expose to AKT agonist SC-79 following *Trps1* deleted. And the different stages of embryonic development were observed in each group. Compared to the KSOM group and negative siRNA group, the transition rate of 2-/4-cell embryo was significantly reduced in *Trps1* siRNA group. In addition, SC-79 treatment could significantly increase the development rate of 4-cell embryo (Fig. 2e, f) (Supplemental Table 3). However, we found that SC-79 treatment alone could not affect embryonic development (Supplemental Fig. 3). Real-Time PCR results suggested that *Akt* and *Creb* mRNA expression were not significantly changed among each group (Fig. 2a). Results of western blot and immunofluorescence indicated that SC-79 could alleviate the decreased phosphorylation levels of AKT and CREB caused by *Trps1* deficiency (Fig. 2b-d). In addition, the results of RT-qPCR indicated that SC-79 could alleviate the decrease of ZGA genes, such as *Chd1*, *Weel*, and *Psmb7*, caused by *Trps1* knockdown (Fig. 2a). The above results suggested that *Trps1* may affect the 2-cell embryo to 4-cell embryo developmental process by regulating AKT and CREB phosphorylation levels.

Trps1 knockdown reduces Pde4d expression in mouse 2-cell embryos

CUT&Tag analysis and ChIP-qPCR indicated that TRPS1 is capable of combination with the promoter of the *Pde4d* (Fig. 3a, b). And *Pde4d* expression was decreased following *Trps1* silencing (Fig. 3c-f). *Wang et al*. have reported that PDE4D facilitates enhanced signaling through the cAMP-PKA-SIRT1-AKT pathway, potentially providing therapeutic advantages in the treatment of neurocognitive disorders (Wang et al. 2020). Therefore, we have a suspicion that *Trps1* may regulate AKT activation through *Pde4d*. Results of immunofluorescence staining showed that PDE4D had high expression in the nucleus of 1-cell and 2-cell embryo, consistent with TRPS1 expression (Liu et al. 2019) (Fig. 4a, b). Decreased of *Pde4d* expression could also down-regulate the phosphorylation levels of AKT and CREB, blocking the development of 2-cell embryos (Fig. 4c, d, Fig. 5) (Supplemental Table 3). In addition, compared with *Pde4d* siRNA microinjection alone, SC-79 treatment could restore the AKT/CREB pathway inhibition caused by *Ped4d* knockdown, thereby promoting 2-/4-cell embryo transition (Fig. 5). It verified that *Pde4d* may be involved in regulating the development of mouse preimplantation embryos through the activation of the AKT/CREB pathway.

Supplementing *Pde4d* alleviates *Trps1* deficiency induced arrest of 2-cell embryos

To confirm the above hypothesis, we further microinjected *Pde4d* mRNA in *Trps1* deficiency embryo to observe the rate of embryo development. Compared with the *Trps1* siRNA group, the transition rate from 2-cell embryos to 4-cell embryo was increased in *Trps1* siRNA + *Pde4d* mRNA group (Fig. 6a, b) (Supplemental Table 3). Western blot and immunofluorescence were also used to observe the expression levels of AKT and CREB proteins in each group. The results demonstrated that compared with the *Trps1*-siRNA injection group, the phosphorylation of AKT and CREB significantly increased in the *Trps1* siRNA + *Pde4d* mRNA group, while there were no significantly changed in AKT and CREB proteins expression (Fig. 6c-e). The above results exhibited that *Trps1* may affect the 2-cell/4-cell embryo transition by regulating the expression of *Pde4d*.

Discussion

Previous reports have shown that ZGA represents the first transcriptional event in new a life and the beginning of the embryonic programming (Svoboda 2018; Zou et al. 2022). However, the regulatory mechanisms of ZGA are still largely unknown in mammals (Gassler et al. 2022). Our previous studies have shown that the transcription factor *Trps1* is essential for the development of preimplantation embryo. Knockdown of *Trps1* in 1-cell embryo would lead to 2-cell embryo arrest (Liu et al. 2019), which implied that *Trps1* may be involved in ZGA process, but its mechanism is still unclear. In this study, we demonstrated that TRPS1 could enhance *Pde4d* transcription to activate AKT/CREB pathway, thereby regulating ZGA process.

Pioneer transcription factors bind to target sequences on nucleosome DNA and are recruited into enclosed chromatin in vivo, triggering local chromatin opening through multiple mechanisms (Iwafuchi-Doi and Zaret 2014). For example, Zelda is one of the important transcription factors that regulate ZGA in Drosophila. It has six C2H2 zinc finger structures and can act as a regulatory factor for the occurrence of ZGA by regulating chromatin accessibility or histone modifications in the binding region (Harrison et al. 2011; Schulz et al. 2015). Although Zelda is not present in animals other than insects (Ribeiro et al. 2017), we believed that transcription factors similar to Zelda may exist in mammals. TRPS1 is a noteworthy atypical GATA transcription factor, similar to the structure of Zelda protein, with six C2H2 zinc finger structures. In tumor research, TRPS1 often acts as a transcription inhibitor to regulate the epithelial mesenchymal transition (EMT) process, thereby affecting the occurrence and progression of tumors (Hu et al. 2018; Huang et al. 2016; Su et al. 2014). In addition, overexpression of *Trps1* was able to promote the proliferation of renal tubular epithelial cells through the PI3K/AKT signaling pathway in a rat model of acute kidney injury (Ju-Rong et al. 2017). In this study, KEGG analysis of enriched genes in TRPS1 CUT&Tag datasets revealed that TRPS1 probably regulates the AKT signaling pathway in 2-cell embryos. Additionally, phosphorylation of AKT and CREB depressed following *Trps1* deleted.

AKT belongs to the serine/threonine protein kinase family, which has been widely identified as expressed in oocytes, embryos, ovaries and uterus, and plays a crucial regulatory role in embryonic development (Riley et al. 2005). It was shown that AKT can regulate the G2/M transition and influence the cell cycle by affecting p21 localization in mouse 1-cell embryo (Wu et al. 2011). Inhibition of AKT in 1-cell embryo would down-regulated the development rate of 4-cell embryo(Chen et al. 2016). And after inhibiting AKT phosphorylation in 8-cell embryos, the expression of the trophoblast ectoderm marker factor CDX2 was decreased and blastocyst formation was also inhibited (Xu et al. 2019). It can be inferred that activation of the AKT pathway is essential for the early mouse embryonic development. Cyclic-AMP response binding protein (CREB) is downstream of AKT, which has transcriptional regulatory functions (Truong et al. 2021). Jin et al. implied that *Creb1* mRNA is expressed at all stages of the preimplantation embryo. And compared with other stages, CREB and p-CREB are most obviously located in the nucleus at 2-cell embryo (Jin and O'Neill 2007). *Bleckmann* et al. indicated that the absence of CREB can lead to embryo death before implantation(Bleckmann et al. 2002). SC-79 acts as an agonist for AKT, enhancing its phosphorylation at the Thr308 and Ser473 sites (Sun et al. 2022). Our results showed that SC-79 could promote the activation of AKT and CREB and improve the rate of development from 2-cell embryos to 4-cell embryos following *Trps1* knockdown. It indicated that *Trps1* may influence mouse ZGA via the AKT/CREB pathway.

In order to explore the mechanism, we focused on the targeted genes of *Trps1* in AKT pathway related genes based on the CUT&Tag datasets, and *Pde4d* was observed in the enriched genes. It has been reported that *Pde4d* is closely related to AKT pathway in cancers. In colorectal cancer, *PDE4D* could regulate cellular malignant progression through AKT/mTOR/Myc pathway (Kim et al. 2019). In nasopharyngeal carcinoma, *PDE4D* targeted phosphorylation levels of AKT and EGFP to affect cell proliferation (Xu et al. 2014). However, the relationship between PDE4D and AKT in early embryonic development remains unreported. In this research, we mentioned that the expression pattern of PDE4D protein was similar to that of TRPS1 (Liu et al. 2019). It was clearly located in the nucleus at 1-/2-/4-cell embryo, and gradually decreased from 8-cell embryo to the blastocyst stage. After *Pde4d* siRNA treatment, the activation of AKT and CREB were inhibited and mouse preimplantation embryo were blocked in 2-cell embryo stage. In addition, AKT agonist SC-79 alleviate *Pde4d* deficiency induced arrest of 2-cell embryo. The results declared that *Pde4d* also regulated ZGA through affecting AKT activation.

To explore whether *Trps1* promotes AKT and CREB phosphorylation through *Pde4d*, we further designed the rescue experiment. The results exhibited that *Pde4d* expression were decreased following *Trps1* knockdown. In addition, *Pde4d* overexpression could rescue inhibition of preimplantation embryo development caused by *Trps1* deficiency. In summary, these results advised that TRPS1 targets *Pde4d* to regulate the phosphorylation of AKT and CREB, thereby affecting ZGA process and mouse preimplantation embryo development.

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Author contributions Shie Wang and Yue Liu contributed to the study conception and design. Material preparation was performed by Xia Jiang, Weiwei Xu, Jiandong Sun, Xiuli Lian and Jianmin Lin. Data collection was performed by Xia Jiang, Weiwei Xu, Jiandong Sun, Xiuli Lian, Jianmin Lin and Zihang Lin. Analysis was performed by Jiang Xia, Weiwei Xu, Shanshan Luo, Shumin Liao and Zihang Lin. The first draft of the manuscript was written by Jiandong Sun and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability CUT &Tag data were deposited in the GEO database with accession number GSE283924.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate This study was approved by the local ethics committee of Fujian Medical University (Code of Ethics: LLSLBH-20210930–002).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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