**Supplementary Materials**

**Atypical GATA protein TRPS1 play indispensable roles in the mouse 2-cell embryo**

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**Figures S1-S5**

Fig S1.tif

**Figure S1. Micro-injection of anti-TRPS1 antibody decreased the development potential of mouse zygotes.** Mouse zygotes were micro-injected with different concentrations of anti-TRPS1 antibody in the nucleus. Chi-square (χ2)-test was performed. \* *P* < 0.05, \*\* *P* < 0.01, \*\*\* *P* < 0.001.

**Fig S2.tifFigure S2. Co-injection of Trps1-siRNA and ERα-siRNA further decreased the development rate of blastocyst.** Mouse zygotes were injected with a mixture of Trps1-siRNA and ERα-siRNA (10 pl in total). Experiment was performed in 3 independent replicates, and total embryo number of each stage (at least 180 cells) was calculated and compared. Chi (χ2)-test was performed. \*\*\* *P* < 0.001.



**Figure S3. Motif analysis revealed multiple ERα and GATA/TRPS1 binding sites on the regulatory regions of Zscan4d and on neighboring repeat element MuERV-L.** JASPAR on-line software analysis was performed with parameter setting as 90%. This figure is corresponding to Supplementary Table S1-S3 (overlapped binding sites are not shown). Upstream of Zscan4d (blue), there is a long MuERV-L element composed of MERVL-int (yellow), ORR1A3-int (light blue) and MT2\_Mm (green). Both intergenic region (thick black line) and MuERV-L element contain multiple binding sites for ESR1 (i.e. ERα) (red dots) and GATA family members including TRPS1 (purple dots).

**Figure S4. Enrichment of H3K4me3 and H3K27me3 on Zscan4d and neighboring MuERV-L.** (A) H3K4me3 and H3K27me3 ChIP-seq RPM (reads per million mapped reads) data of mouse 2-cell, 4-cell and 8-cell were extracted from GSE73952 (Liu et al., 2016), and visualized by using UCSC genome browser. (B) Stage-specific Zscan4d RNA-seq data (FPKM, fragments per kilobase of transcript per million mapped reads) extracted from GSE71434 (Zhang et al., 201Fig-S4.tif6).

Fig-S5.tif

**Figure S5. Neither H3K27ac nor H3K36me3 expression was changed after Trps1 knockdown.** (A) H3K27ac ChIP-seq data of mouse 2-cell and 8-cell embryo were extracted from GSE66390 (Wu et al., 2016), and visualized by using UCSC genome browser. (B to E) Effects of Trps1 knockdown on the expression of H3K27ac (B, C) and H3K36me3 (D, E). Mid 2-cell, 20 h post injection; late 2-cell, 29 h post injection. About 80 embryos were used in each experimental group (N=3). Student’s *t*-test. Error bars indicate SEM. Scale bar, 20 μm.

*Fig-S6.tif*

**Figure S6. Trps1 knockdown did not change the expression level of NANOG.** (A to C) Immunofluorescence staining showed the effects of Trps1-siRNA on the expression of NANOG, gray values were analyzed by using SmartScape software. Mid 2-cell, 20 h post injection; late 2-cell, 29 h post injection. About 80 embryos were used in each experimental group (N=3). Student’s *t*-test. Error bars indicate SEM. Scale bar, 20 μm.

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| **Table S1. ESR1 and GATA motif analysis on promoter regions (-2.5kb of TSS) of Zscan4d, eIF1A and Hsp 70.1 (parameter setting: 90%)** | | | | | | | |
| **Target genes** | **Model name** | **Score** | **Relative score** | **Start** | **End** | **Strand** | **predicted site sequence** |
| Zscan4d | Gata4 | 9.4 | 0.90693646 | 1865 | 1875 | 1 | ccctatcttct |
| ESR1 | 18.955 | 0.901574362 | 2240 | 2259 | 1 | cccctaggtcagactgacct |
| ESR1 | 21.026 | 0.933008709 | 2245 | 2264 | -1 | atccaaggtcagtctgacct |
| eIF1A | / | / | / | / | / | / | / |
| Hsp 70.1 | Gata4 | 9.427 | 0.907324648 | 1243 | 1253 | -1 | ccttctctgcc |

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| **Table S2. ESR1 and GATA motif analysis on DNA sequence between Zscan4d and upstream MuERV-L (parameter setting: 90%) Location: chr7: 11751498-11755788** | | | | | | |
| **Model name** | **Score** | **Relative score** | **Start** | **End** | **Strand** | **predicted site sequence** |
| Gata4 | 11.314 | 0.934454633 | 385 | 395 | -1 | ccttatctgga |
| GATA2 | 14.128 | 0.944214815 | 386 | 399 | -1 | agatccttatctgg |
| Gata1 | 14.05 | 0.960335909 | 386 | 396 | -1 | tccttatctgg |
| GATA3 | 13.374 | 0.97943511 | 388 | 395 | 1 | agataagg |
| Gata4 | 11.314 | 0.934454633 | 407 | 417 | -1 | ccttatctgga |
| GATA2 | 11.91 | 0.913975035 | 408 | 421 | -1 | tgacccttatctgg |
| Gata1 | 12.986 | 0.945757307 | 408 | 418 | -1 | cccttatctgg |
| GATA3 | 13.374 | 0.97943511 | 410 | 417 | 1 | agataagg |
| Gata4 | 10.814 | 0.927265978 | 1421 | 1431 | -1 | tgttatcacct |
| GATA2 | 11.278 | 0.90535847 | 1422 | 1435 | -1 | caaatgttatcacc |
| Gata4 | 9.4 | 0.90693646 | 3658 | 3668 | 1 | ccctatcttct |
| ESR1 | 18.955 | 0.901574362 | 4033 | 4052 | 1 | cccctaggtcagactgacct |
| ESR1 | 21.026 | 0.933008709 | 4038 | 4057 | -1 | atccaaggtcagtctgacct |

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| **Table S3. ESR1 and GATA motif analysis on Zscan4d upstream MuERV-L (parameter setting: 90%) Location: chr7: 11755789-11761733** | | | | | | |
| **Model name** | **Score** | **Relative score** | **Start** | **End** | **Strand** | **predicted site sequence** |
| Gata4 | 11.314 | 0.934454633 | 383 | 393 | -1 | ccttatctgga |
| GATA2 | 14.128 | 0.944214815 | 384 | 397 | -1 | agatccttatctgg |
| Gata1 | 14.05 | 0.960335909 | 384 | 394 | -1 | tccttatctgg |
| GATA3 | 13.374 | 0.97943511 | 386 | 393 | 1 | agataagg |
| Gata4 | 11.314 | 0.934454633 | 405 | 415 | -1 | ccttatctgga |
| GATA2 | 11.91 | 0.913975035 | 406 | 419 | -1 | tgacccttatctgg |
| Gata1 | 12.986 | 0.945757307 | 406 | 416 | -1 | cccttatctgg |
| GATA3 | 13.374 | 0.97943511 | 408 | 415 | 1 | agataagg |
| Gata4 | 9.832 | 0.913147459 | 5535 | 5545 | -1 | tcttctctctt |
| Gata4 | 10.245 | 0.919085288 | 5607 | 5617 | 1 | ttttatcacac |

**Supplementary Materials and Methods**

**Supplement Reagents**

ERα Mouse Monoclonal antibody (Millipore, Germany); Normal Rabbit IgG (Beyotime, China); H3K27ac Rabbit Monoclonal antibody (Cell Signaling, USA); H3K36me3 Rabbit Monoclonal antibody (Cell Signaling, USA); NANOG Rabbit Monoclonal antibody (Cell Signaling, USA); Alexa Fluor®488 Labeled Donkey anti-Rabbit secondary antibody (Life Technology, USA); ERα-siRNA (Santa Cruz, USA); Negative control siRNA (sense: 5’-UUCUCCGAACGUGUCACGUTT-3’; antisense: 5’-ACGUGACACGUUCGGAGAATT-3’) (CenePharma, China).

**Antibody micro-injection of mouse zygotes**

Micro-injection procedure is described in Materials and Methods. Anti-TRPS1 antibody was diluted with KSOM to concentrations of 0.03 μg/μl, 0.1 μg/μl, 0.3 μg/μl. Rabbit IgG was diluted to 0.3 μg/μl with KSOM medium.