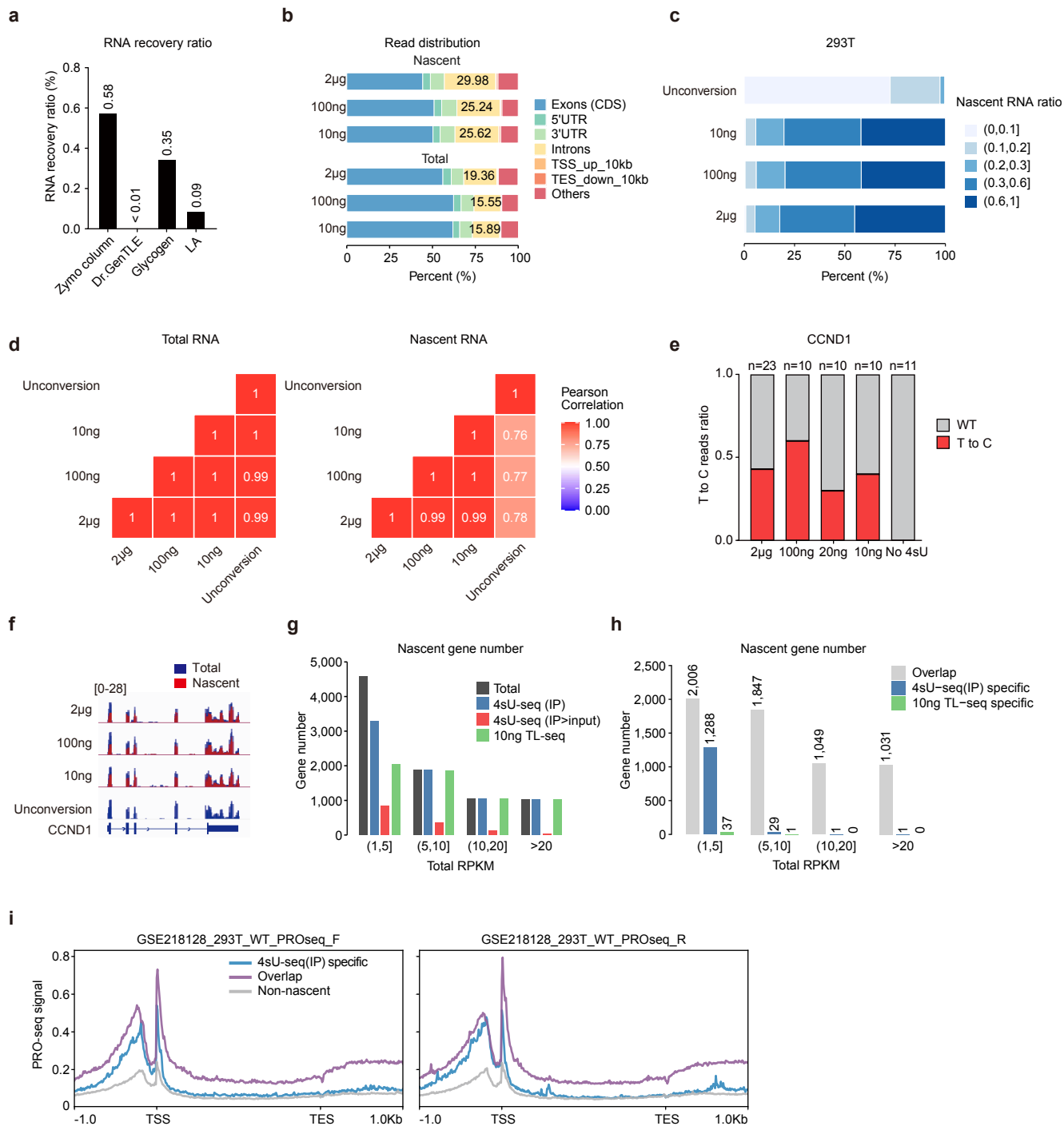
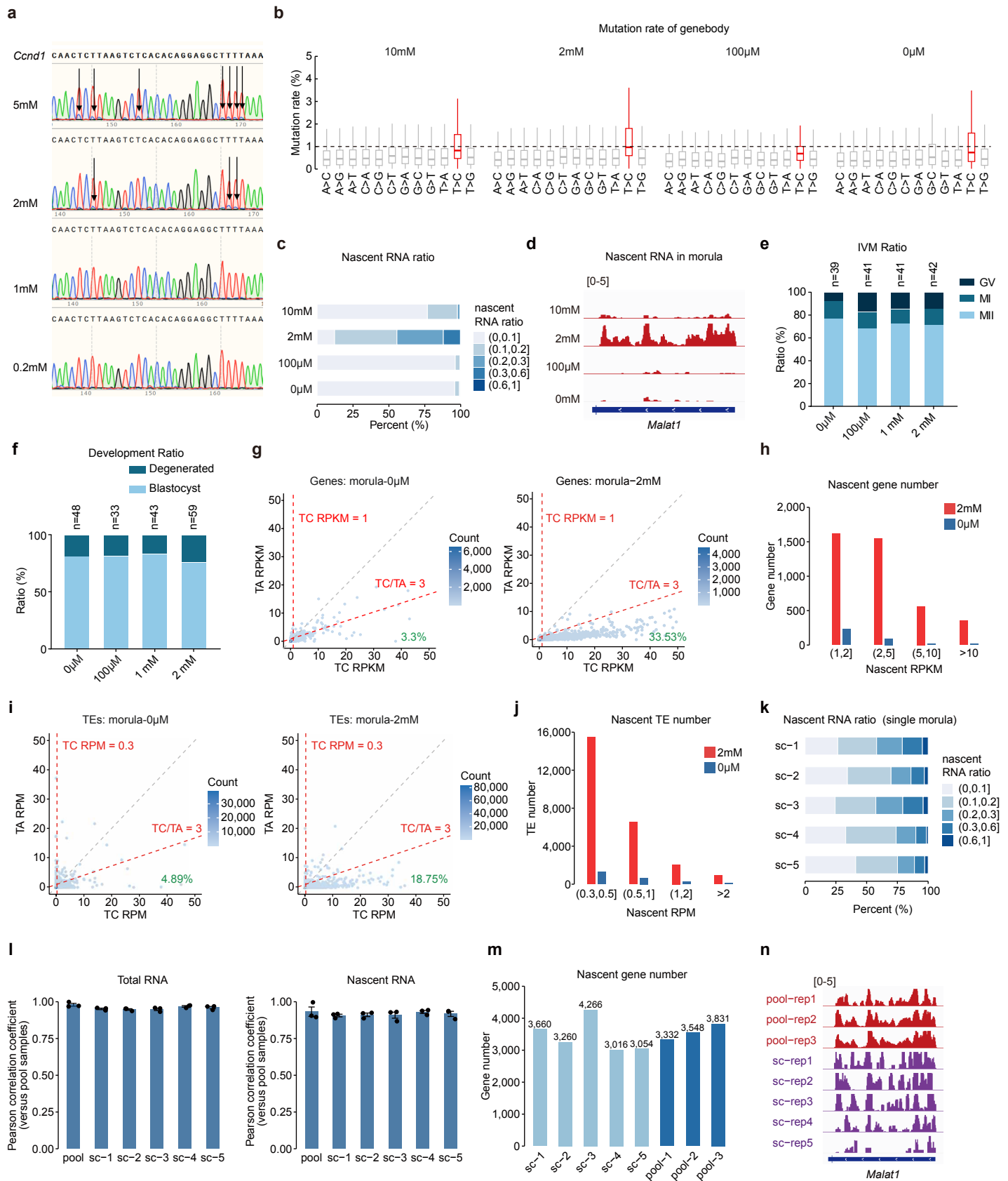




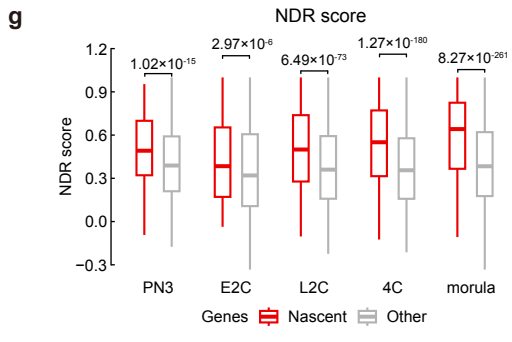
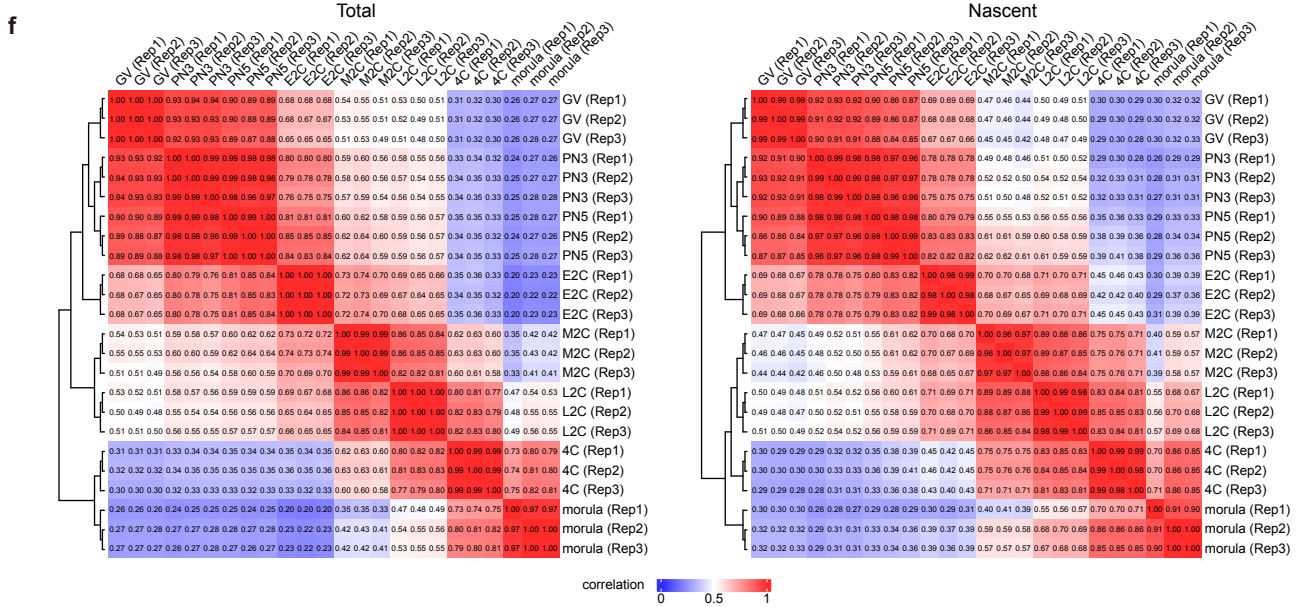
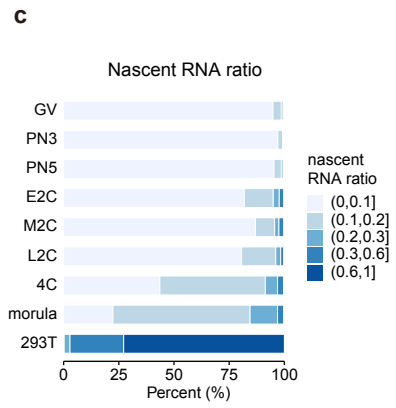
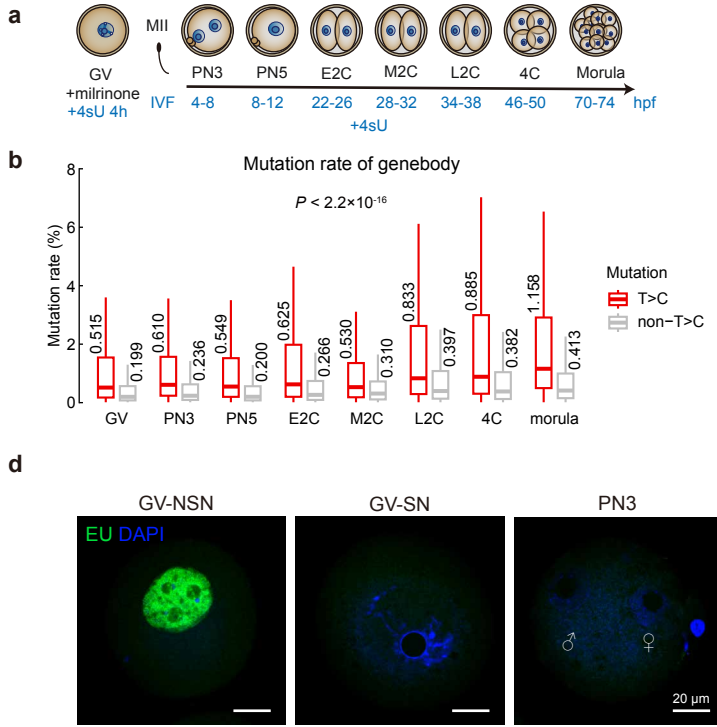
Supplementary Fig.1 | relative to Fig. 1

- a.** RNA recovery ratio after TimeLapse conversion reaction using different extraction methods. Zymo column, RNA extracted using zymo RNA purification kit. Dr.GenTLE, glycogen, and LA were different RNA precipitants.
- b.** Reads distribution of nascent transcripts and total transcripts in 293T cells with different amounts of RNA for TimeLapse-seq.
- c.** Nascent RNA ratio of different amounts of RNA compared to the unconverted sample. Ratio groups represent the ratio of nascent RNA versus total RNA.
- d.** Pearson correlations among samples with different RNA input amounts and the unconverted sample, based on read counts of total RNA (left) and nascent RNA (right) for all protein-coding genes.
- e.** T-to-C reads ratio in *CCND1* tested by TimeLapse-seq and Sanger sequencing with different amounts of RNA prepared from 293T cells.
- f.** Genome browser tracks showing total and nascent TimeLapse-seq read distribution of *CCND1* with different amounts of RNA prepared from 293T cells. Blue reads represent total reads; red reads represent nascent reads.
- g.** Number of nascent genes in different RPKM groups identified using TimeLapse-seq and 4sU-seq.
- h.** Number of overlapping and method-specific nascent genes identified by TimeLapse-seq and 4sU-seq (IP) across different RPKM ranges.
- i.** PRO-seq signal intensity profiles (-1 kb of TSS to +1 kb of TES) for overlapping nascent genes, 4sU-IP specific nascent genes and non-nascent genes in 293T cells (data from GES218128). Left panel: forward sequences PRO-seq. Right panel: reverse sequences of PRO-seq.



Supplementary Fig.2 | relative to Fig. 1

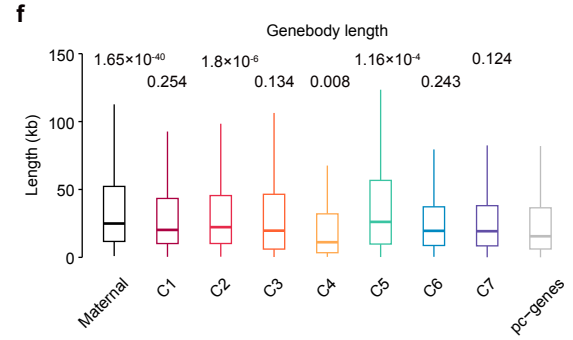
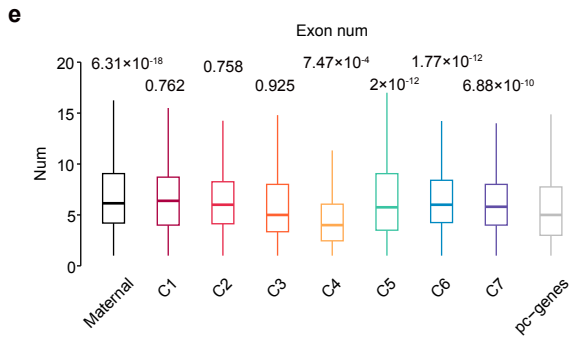
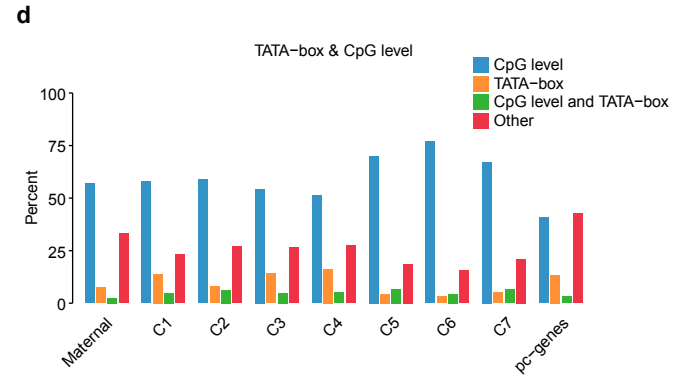
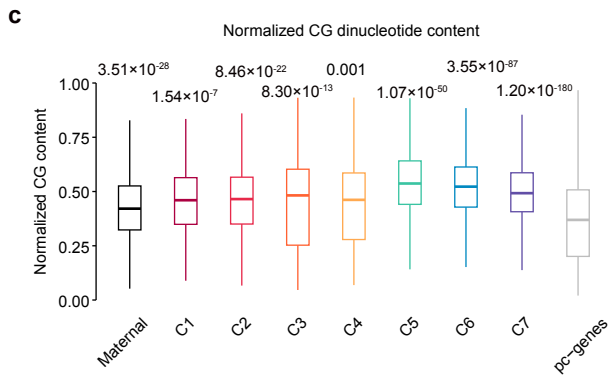
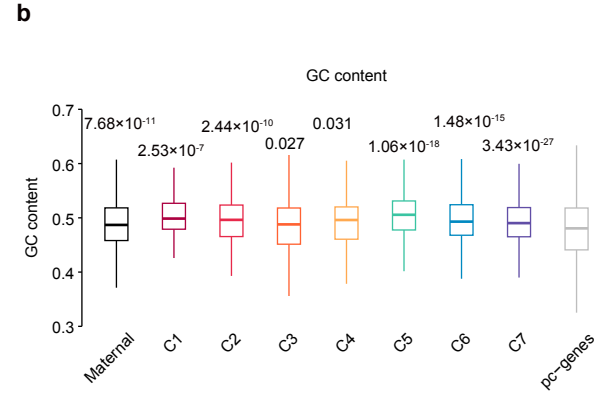
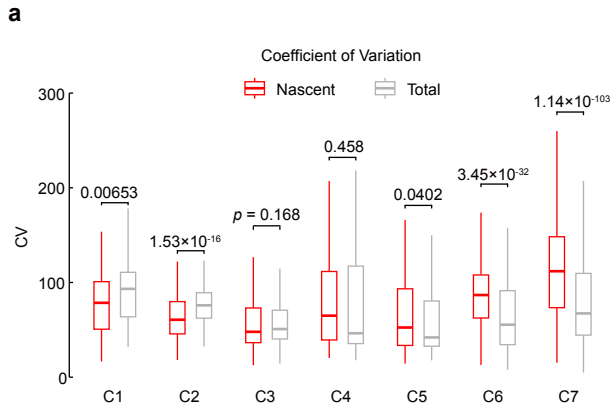
- a.** T-to-C mutation sites within *Ccnd1* transcripts detected by Sanger sequencing after reverse-transcription PCR from mouse ESCs treated with different 4sU concentrations. Arrowheads indicate a C peak beneath the T peak, representing T-to-C conversion.
- b.** Mutation rates after TimeLapse-seq with different concentrations of 4sU labeling for 4h in morula. In the box plots, the centre line indicates the median; the box bounds represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; and the whiskers extend to $Q3 + 1.5 \times IQR$ (upper) and $Q1 - 1.5 \times IQR$ (lower), where $IQR = Q3 - Q1$.
- c.** Nascent RNA ratio with different concentrations of 4sU labeling for 4h in morula. The color bar groups represent the ratio of nascent RNA to total RNA.
- d.** Genome browser tracks showing nascent read distribution for *Malat1* in morula labeled with different 4sU concentrations.
- e.** Maturation rates of GV oocytes treated with different 4sU concentrations for 4 hours followed by 24 hours of in vitro maturation. n: number of oocytes per group. Exact *P* values are provided in Source data.
- f.** Blastocyst rates of zygotes treated with different 4sU concentrations for 4 hours and then cultured to the blastocyst stage. n: number of embryos per group. Exact *P* values are provided in Source data.
- g.** T-to-C(TC) and T-to-A(TA) reads level of each gene (RPKM) in morula samples with 0 μ M 4sU (left) and 2 mM 4sU treatment (right). Grey dashed line represents TC/TA=1. Red dashed line represents TC=1 and TC/TA=3, green number represents gene percentage with TC>1 and TC/TA>3.
- h.** Number of nascent genes in different RPKM groups using the cutoff: TC>1 and TC/TA>3.
- i.** TC and TA reads level of each TE copy (RPM) in morula samples with 0 μ M 4sU (left) and 2 mM 4sU treatment (right). Grey dashed line represents TC/TA=1. Red dashed line represents TC=0.3 and TC/TA=3, green number represents TE percentage with TC>0.3 and TC/TA>3.
- j.** Number of nascent TEs in different RPM groups using the cutoff: TC>0.3 and TC/TA>3.
- k.** Nascent RNA ratio in single morula. Ratio groups represent the ratio of nascent RNA versus total RNA.
- l.** Pearson correlation of total RNA (left) and nascent RNA (right) between single morula and pooled morulae samples. Read counts of all protein-coding genes were used to calculate the Pearson correlation. *p* -values from Student t-test are shown. n=3 biological replicates.
- m.** Number of nascent genes identified by scTL-seq in pooled and single morula samples.
- n.** Genome browser tracks showing nascent reads coverage for *Malat1* in pooled and single morula samples.



Supplementary Fig.3 | relative to Fig. 1

- a.** Schematic diagram showing the embryo collection time points for scTL-seq.
- b.** Rates for T-to-C and other (non-TC) mutations in gene bodies after scTL-seq.
- c.** Average nascent RNA ratios at each development stage. Ratio groups represent the ratio of nascent RNA versus total RNA.
- d.** EU incorporation in NSN and SN GV oocytes and PN3 1-cell embryos. Scale bar = 20µm.
- e.** Quantification of EU signal intensity in nuclei of GV and 1C embryos. Bars are represented as mean values $\pm$ SD; dot points display individual replicate values of nuclei; *P* value are from one-tailed unpaired Student *t*-test.
- f.** Heatmaps showing Pearson correlations among scTL-seq samples based on read counts of total RNA (left) and nascent RNA (right) for all protein-coding genes.
- g.** Nucleosome Depletion Region (NDR) score from MNase-seq at distinct stages.

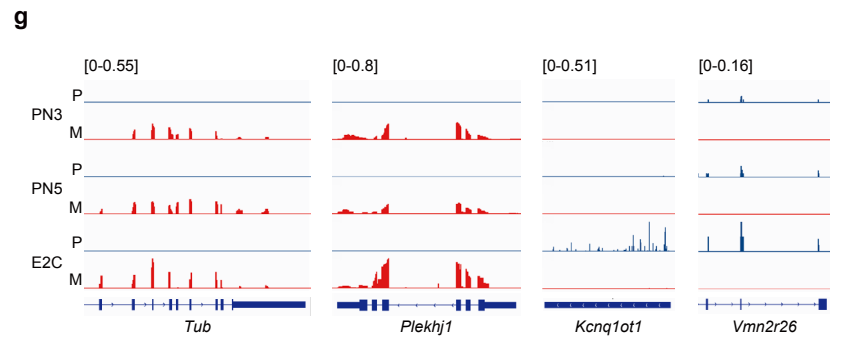
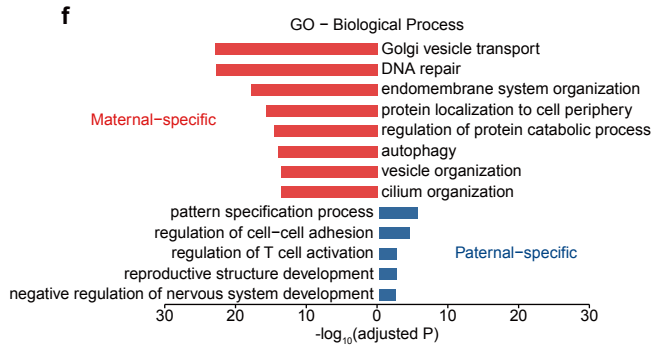
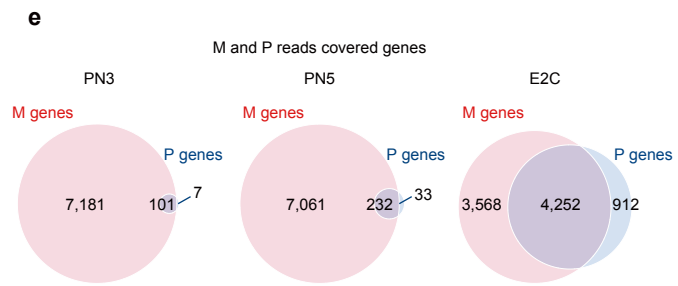
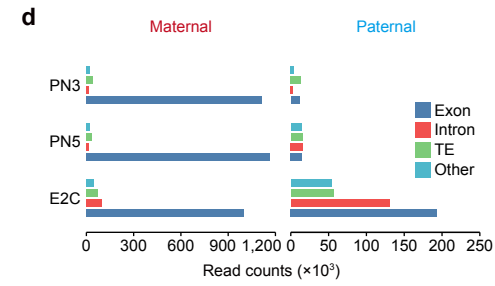
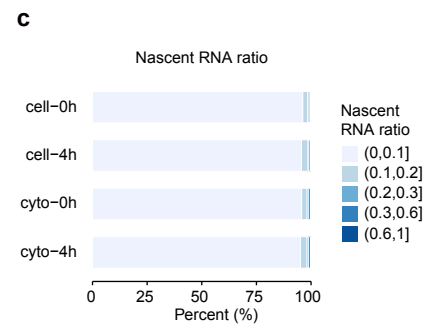
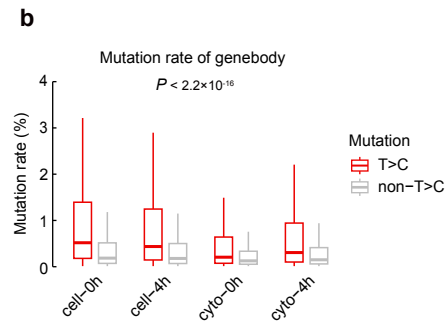
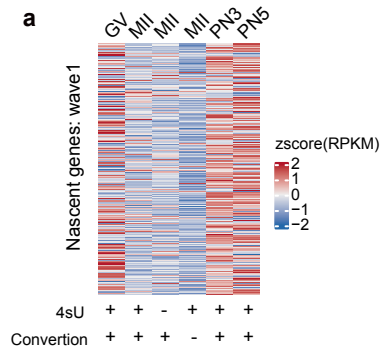
For panels **b** and **g**, *P* values from two-sided Wilcoxon rank-sum tests are shown, and the exact sample sizes are provided in Supplementary Table 5. Extremely small *P* values are reported as $P < 2.2 \times 10^{-16}$ owing to the computational precision limit of R. In the box plots, the centre line indicates the median; the box bounds represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; and the whiskers extend to $Q3 + 1.5 \times \text{IQR}$ (upper) and $Q1 - 1.5 \times \text{IQR}$ (lower), where $\text{IQR} = Q3 - Q1$.



Supplementary Fig.4 | relative to Fig. 2

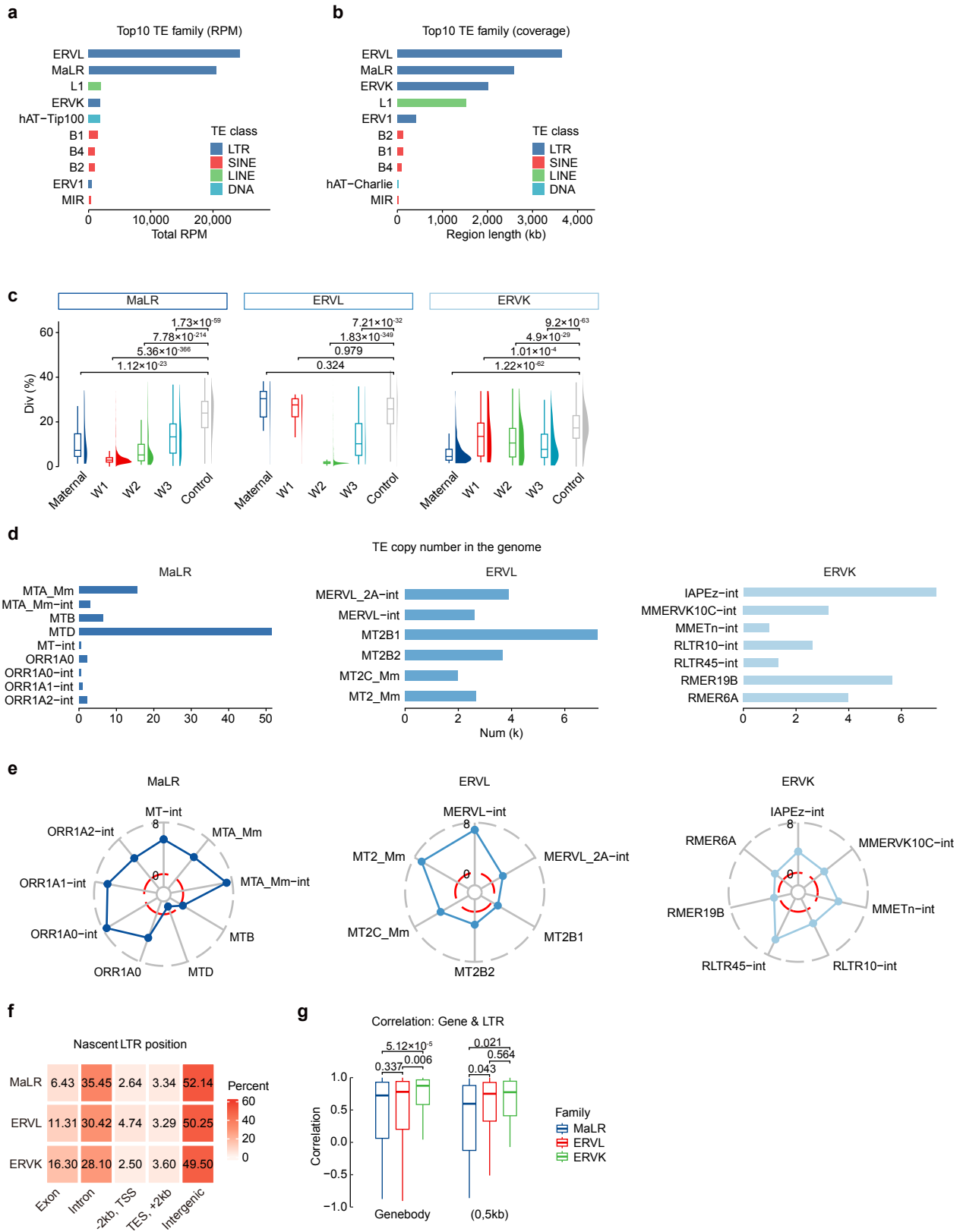
- a.** Coefficient of variation (CV) of total and nascent expression levels for each cluster.
- b.** CG content at promoter regions (defined as 2 kb upstream and downstream of TSSs) for each cluster compared with all protein-coding genes.
- c.** Normalized CG dinucleotide content at promoter regions for each cluster compared with all protein-coding genes.
- d.** TATA-box and CpG island presence at the transcription start site (TSS) for each cluster and all protein-coding genes. The red bar represents genes lacking both TATA-box and CpG islands at their TSSs.
- e.** Number of exons per isoform for each cluster compared with all protein-coding genes.
- f.** Gene body length for each cluster compared with all protein-coding genes.

For panels **a**, **b**, **c**, **e** and **f**, *P* values from two-sided Wilcoxon rank-sum tests are shown, and the exact sample sizes are provided in Supplementary Table 5. In the box plots, the centre line indicates the median; the box bounds represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; and the whiskers extend to $Q3 + 1.5 \times IQR$ (upper) and $Q1 - 1.5 \times IQR$ (lower), where $IQR = Q3 - Q1$.



Supplementary Fig.5 | relative to Fig.2

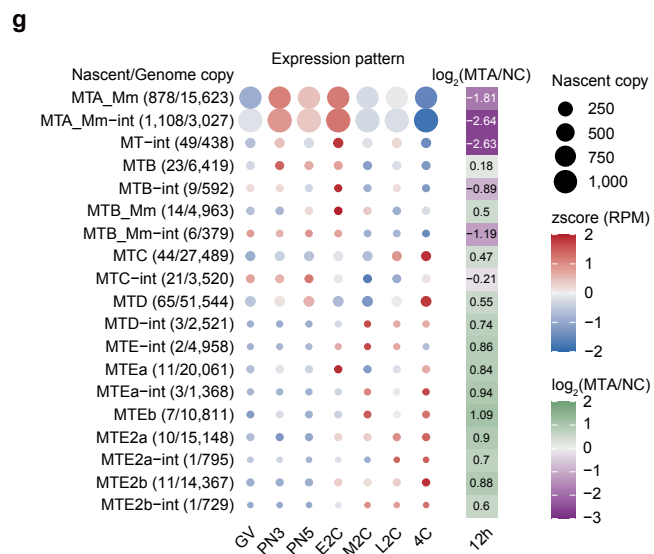
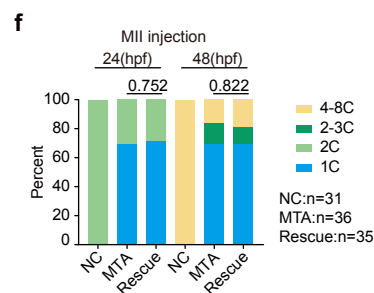
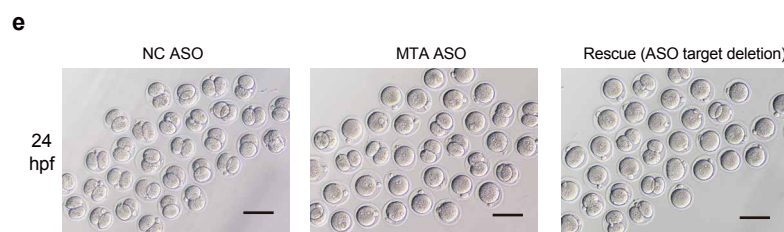
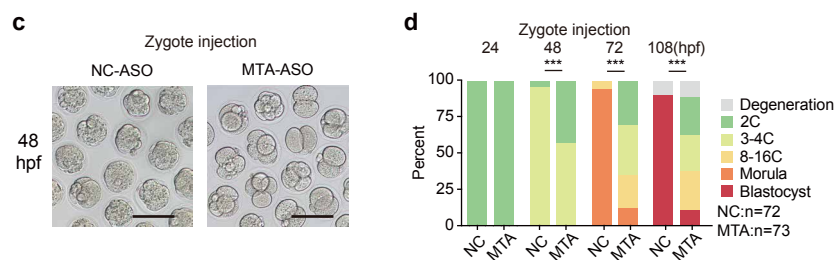
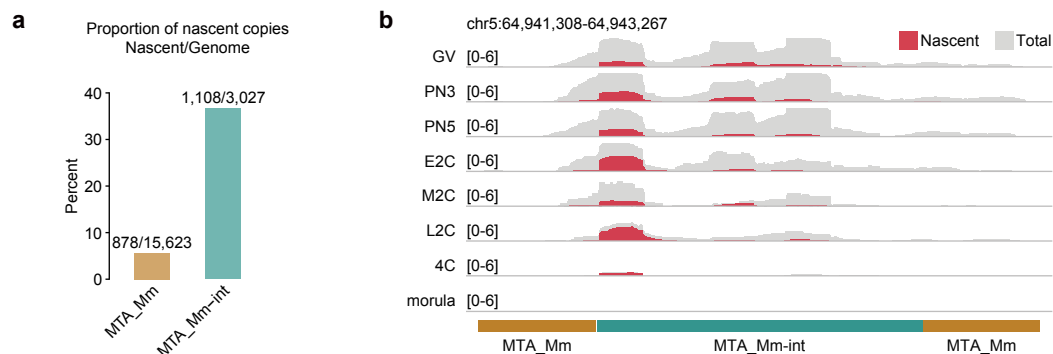
- a.** Heatmap showing nascent RNA levels (nascent RPKM) of wave-1 genes across indicated MII control groups and other developmental stages.
- b.** Rates for T-to-C and other (non-TC) mutations in the gene body of the whole embryo and cytoplasm samples after scTL-seq. Extremely small P values are reported as $P < 2.2 \times 10^{-16}$ owing to the computational precision limit of R. In the box plots, the centre line indicates the median; the box bounds represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; and the whiskers extend to $Q3 + 1.5 \times IQR$ (upper) and $Q1 - 1.5 \times IQR$ (lower), where $IQR = Q3 - Q1$.
- c.** Nascent RNA ratio of the whole embryo and cytoplasm samples. Ratio groups represent the ratio of nascent RNA versus total RNA.
- d.** Bar plot showing the classification of nascent maternal and paternal reads mapped to the genome.
- e.** Venn plot showing the overlap of genes covered by M and P reads of each stage.
- f.** GO analysis showing representative biological process terms of maternal and paternal-specific genes across three stages.
- g.** Genome browser tracks showing maternal and paternal read distribution for representative parentally specific genes (merged signal of 3 biological replicates).



Supplementary Fig.6 | relative to Fig. 3

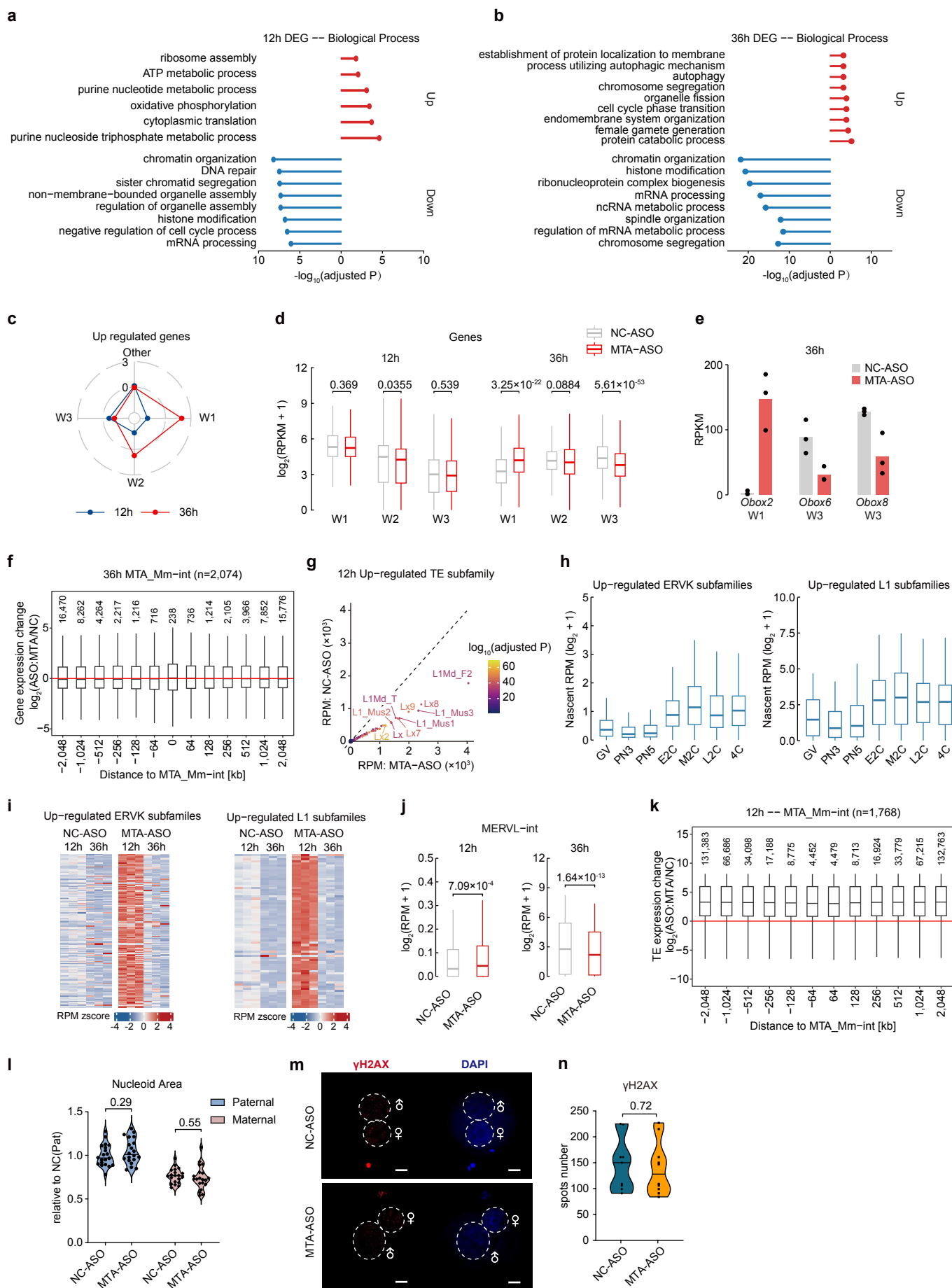
- a.** Top 10 nascent TE families ranked by TE expression level (RPM, reads per million mapped reads).
- b.** Top 10 nascent TE families ranked by genome coverage.
- c.** Sequence divergence (div) of ERVK, MaLR, and ERVL in each wave, compared with 1000 random copies.
- d.** Genomic copy numbers of LTR subfamilies with high proportions of nascent copies.
- e.** Radar plot showing the enrichment levels of LTR subfamilies with high proportion of nascent copies.
- f.** Genomic distribution of nascent LTR families.
- g.** Box plot showing the correlation between nascent expression levels of genes and nearby LTRs within 5kb.

For panels **c** and **g**, P values from two-sided Wilcoxon rank-sum tests are shown, and the exact sample sizes are provided in Supplementary Table 5. In the box plots, the centre line indicates the median; the box bounds represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; and the whiskers extend to $Q3 + 1.5 \times IQR$ (upper) and $Q1 - 1.5 \times IQR$ (lower), where $IQR = Q3 - Q1$.



Supplementary Fig.7 | relative to Fig. 4

- a.** Nascent copy numbers versus genomic copy numbers of MTA_Mm and MTA_Mm-int.
- b.** IGV snapshot showing an example of a transcriptionally active full-length MTA element (LTR-int-LTR).
- c.** Representative phase-contrast images of embryos from NC and MTA-ASO groups with Zygote injection. Scale bars, 100 μ m.
- d.** Percentage of embryo types at different time points after IVF in NC (n=72) and MTA-ASO (n=73) groups with zygote injection. *P* values are calculated by chi-square test. *** *p* < 0.001. Exact *P* values are in the source data.
- e.** Representative phase-contrast images of embryos at 24 h post-fertilization from NC, MTA-ASO and rescue (Δ ASO target) groups with MII injection. Scale bars, 100 μ m.
- f.** Developmental rates of NC, MTA-ASO, and rescue groups at 24 h and 48 h post-fertilization. *P* values are from chi-square test.
- g.** Expression patterns and knockdown levels of MT family members. Left numbers indicate nascent and genomic copy numbers. Bubble plot shows expression changes at different developmental stages (bubble size represents nascent copy number). Right heatmap shows RNA fold-change after MTA-ASO treatment at 12 h.



Supplementary Fig.8 | relative to Fig. 4

a and b. Gene ontology of differential expressed genes (DEGs) at 12 hpf (a) and 36 hpf (b) after MTA-ASO when MII injection.

c. Rader plot showing enrichment of up-regulated genes in W1-3 at 12 and 36 hpf after MTA-ASO when MII injection.

d. Boxplot showing expression levels of W1-3 genes at 12 and 36 hpf in NC-ASO and MTA-ASO samples when MII injection.

e. Expression levels of *Obox* family genes in different ZGA waves in NC-ASO and MTA-ASO samples at 36 hpf (3 biological replicates).

f. Boxplot showing expression changes for the genes within indicated distances to the downregulated MTA_Mm-int ($\log_2[\text{ASO}/\text{NC}] < -1$, $n = 2074$) upon MTA ASO at 36h (hpf) after MII injection. The number of expressed genes (RPKM >1 in NC-ASO or MTA-ASO samples) in each distance category is annotated above the boxes.

g. Total RNA levels of up-regulated LINE1(L1) subfamilies in NC-ASO and MTA-ASO samples at 12 hpf.

h. Nascent RNA level of up-regulated ERVK (left) and L1(right) subfamilies in WT embryos during ZGA.

i. Heatmap showing relative expression level of up-regulated ERVK (left) and L1 (right) subfamilies in NC-ASO and MTA-ASO samples at 12 and 36 hpf.

j. RNA levels of MERVL-int in NC-ASO and MTA-ASO samples.

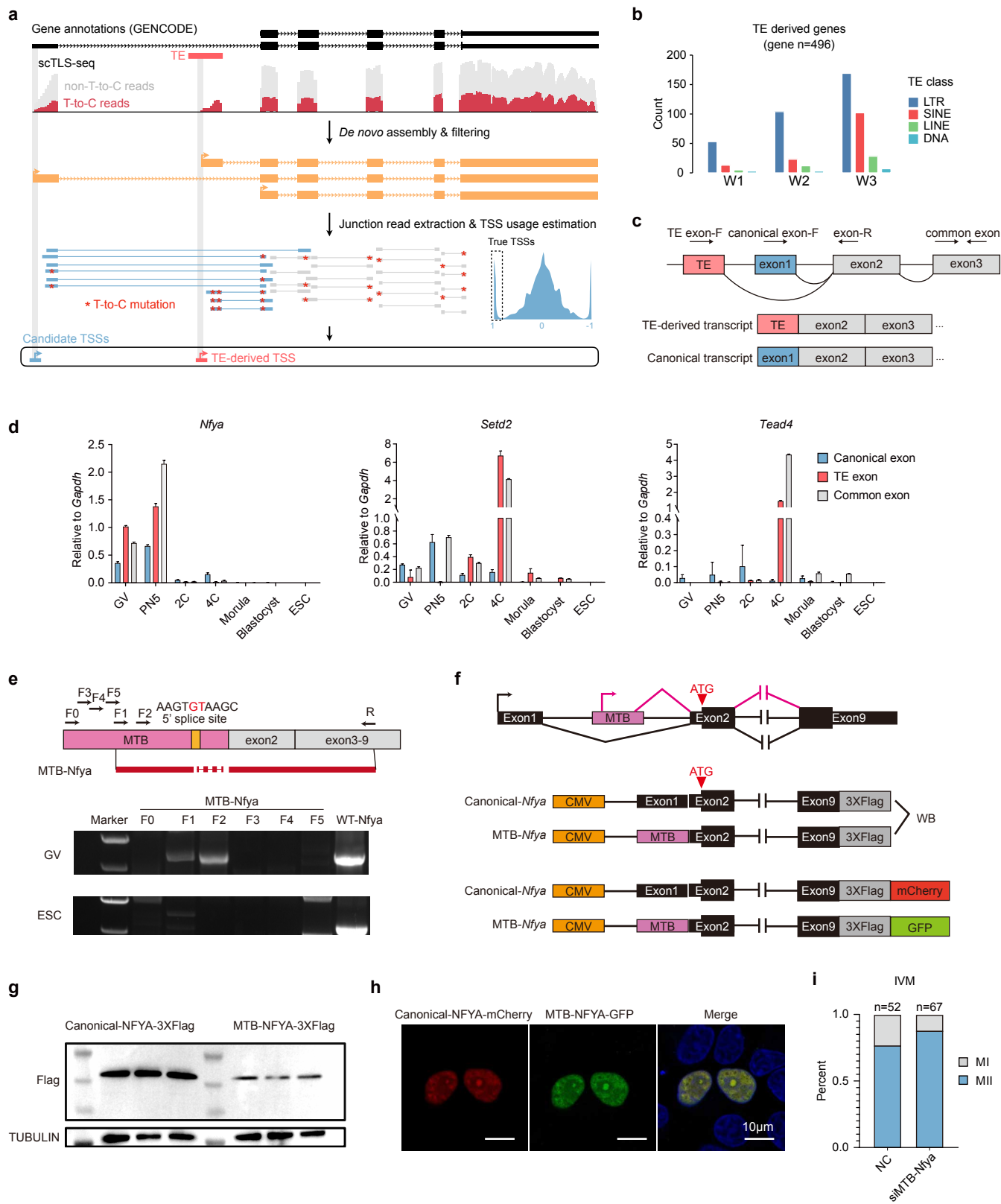
k. Boxplot showing expression changes for the TEs (MT families excluded) within indicated distances to the downregulated MTA_Mm-int ($\log_2[\text{ASO}/\text{Ctrl}] < -1$, $n = 1768$) upon MTA-ASO at 12hpf after MII injection. The number of expressed TEs (RPM >0.3 in Ctrl or ASO samples) in each distance category is annotated above the boxes.

l. Nucleoid area of paternal and maternal pronuclei at 12 hpf after MTA-ASO injection. Violin plot are represented as mean values; dot points are represented as individual values of pronuclei; n (NC) = 20, n (MTA) = 20; P values from two-tailed unpair Student t-test.

m. immunofluorescence images of NC-ASO and MTA-ASO pronuclei at 12 hpf with MII injection. Scale bar= 20 μM .

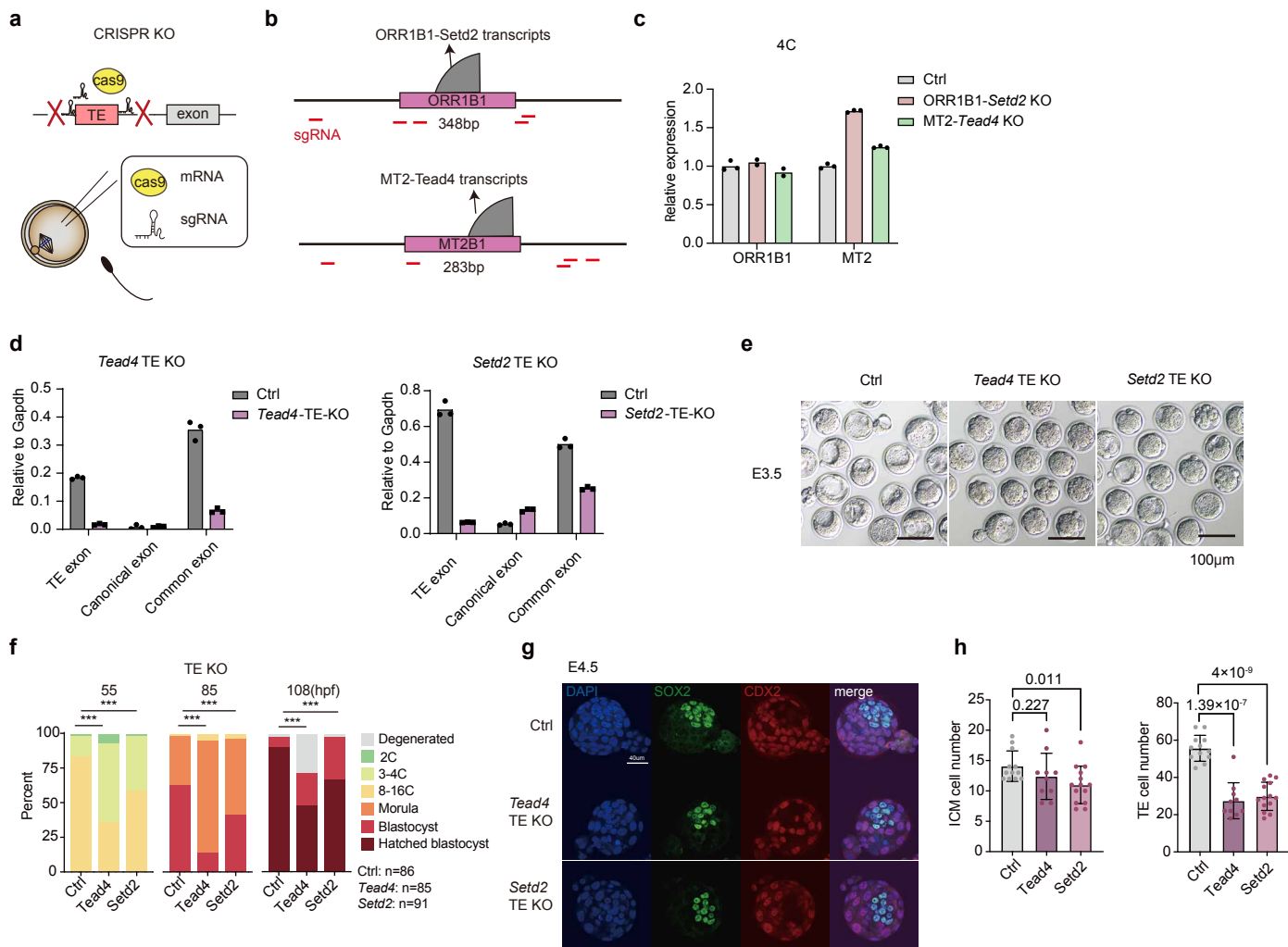
n. γH2AX spots number in NC-ASO and MTA-ASO pronuclei at 12 hpf with MII injection. Violin plot are represented as mean values; dot points are represented as individual values of embryos; n (NC) = 9, n (MTA) = 10; P values from two-tailed unpair Student t-test.

For panels **d**, **f**, **j** and **k**, P values from two-sided Wilcoxon rank-sum tests are shown, with exact sample sizes provided in Supplementary Table 5. In the box plots, the centre line indicates the median; the box bounds represent the first (Q1, 25th percentile) and third (Q3, 75th percentile) quartiles; and the whiskers extend to $Q3 + 1.5 \times \text{IQR}$ (upper) and $Q1 - 1.5 \times \text{IQR}$ (lower), where $\text{IQR} = Q3 - Q1$.



Supplementary Fig.9 | relative to Fig. 5

- a.** Schematic diagram illustrating the *de novo* identification of TE-derived TSSs using scTL-seq data from early embryos.
- b.** Count of genes identified with TE-derived TSSs in three waves.
- c.** Schematic diagram depicting TE-derived transcripts and canonical transcripts. Specific qPCR primer was designed to distinguish TE-derived exon, canonical exon, and common exon.
- d.** Expression levels of indicated transcripts relative to *Gapdh* during ZGA, as measured by qPCR.
- e.** RT-PCR products obtained with different primer sets targeting MTB-Nfya, and the schematic alignment of Sanger sequencing results confirming the splice junction.
- f.** Schematic diagram of the splicing patterns and predicted transcription start sites (red arrow, ATG) of canonical *Nfya* and MTB-*Nfya*, along with the expression vector design.
- g.** Western blot showing the protein sizes produced by the canonical *Nfya* and MTB-*Nfya* expression constructs in human 293T cells.
- h.** Fluorescence reporter assays showing that both canonical NFYA and MTB-NFYA exhibit the same nuclear localization pattern in 293T cells.
- i.** MI and MII ratio of NC and siMTB-Nfya after IVM 16h, number of embryos (n) is shown above the bars.



Supplementary Fig.10 | relative to Fig. 5

- a.** Schematic diagram of experimental procedure for indicated TE KO through CRISPR cas9 editing in early embryos.
- b.** Schematic of the ORR1B1-Setd2 and MT2-Tead4 chimeric transcripts, showing sgRNA numbers and target positions.
- c.** Expression levels of universal ORR1B1 and MT2 elements in 4-cell embryos after ORR1B1-Setd2 KO and MT2-Tead4 KO, respectively. Bars are represented as mean values; dot points are represented as RT-qPCR values from 3 technical replicates.
- d.** Expression levels of indicated transcripts relative to control (only sgRNA without Cas9) after CRISPR KO, measured by qPCR. Bars are represented as mean values; dot points are represented as RT-qPCR values from 3 technical replicates.
- e.** Representative images of embryos at 3.5 dpc (E3.5) after sgRNA and Cas9 mRNA injection in MII oocytes and IVF. Scale bars, 100 μ m.
- f.** Percentage of embryo stages at different time points after IVF in control (n = 86), *Tead4* TE KO (n = 85), and *Setd2* TE KO (n = 91). *P* values are from chi-square test, *** $p < 0.001$. Exact *P* values: Ctrl vs *Setd2*-TE KO: $P(55 \text{ hpf})=1.23\text{E-}10$, $P(85 \text{ hpf})= 3.74\text{E-}05$, $P(108 \text{ hpf})=4.77\text{E-}19$; Ctrl vs *Tead4*-TE-KO: $P(55 \text{ hpf})=7.28\text{E-}32$, $P(85 \text{ hpf})=6.88\text{E-}20$, $P(108 \text{ hpf})= 3.51\text{E-}64$.
- g.** Representative immunofluorescence images of 4.5 dpc (E4.5) blastocysts in control and indicated TE KO. Scale bars, 40 μ m.
- h.** Cell number of ICM (left) and TE (right) indicated by SOX2 positive cells in 4.5 dpc blastocysts of control (n = 12), *Tead4* TE KO (n=10), and *Setd2* TE KO (n = 14). Bars are represented as mean values $\pm$ SEM; dot points are represented as individual values. *P* values from two-tailed unpaired Student t-test are shown.