Supplementary materials for

Reproductive aging causes precocious ZGA and morula gene expression and chromatin hyper-activation in rhesus monkey embryos via maternal SETDB1 insufficiency

Jie Jin *et al*

Corresponding author: Ping Zheng, zhengp@mail.kiz.ac.cn

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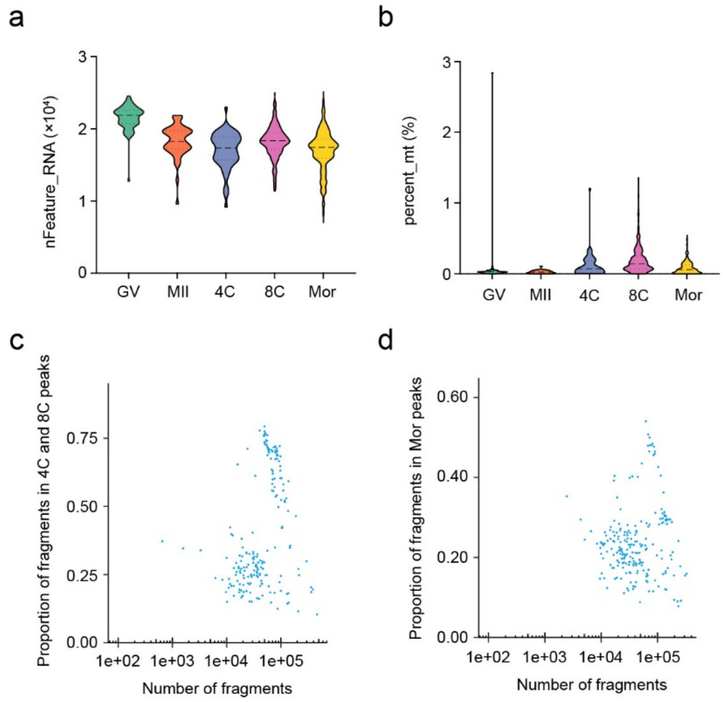


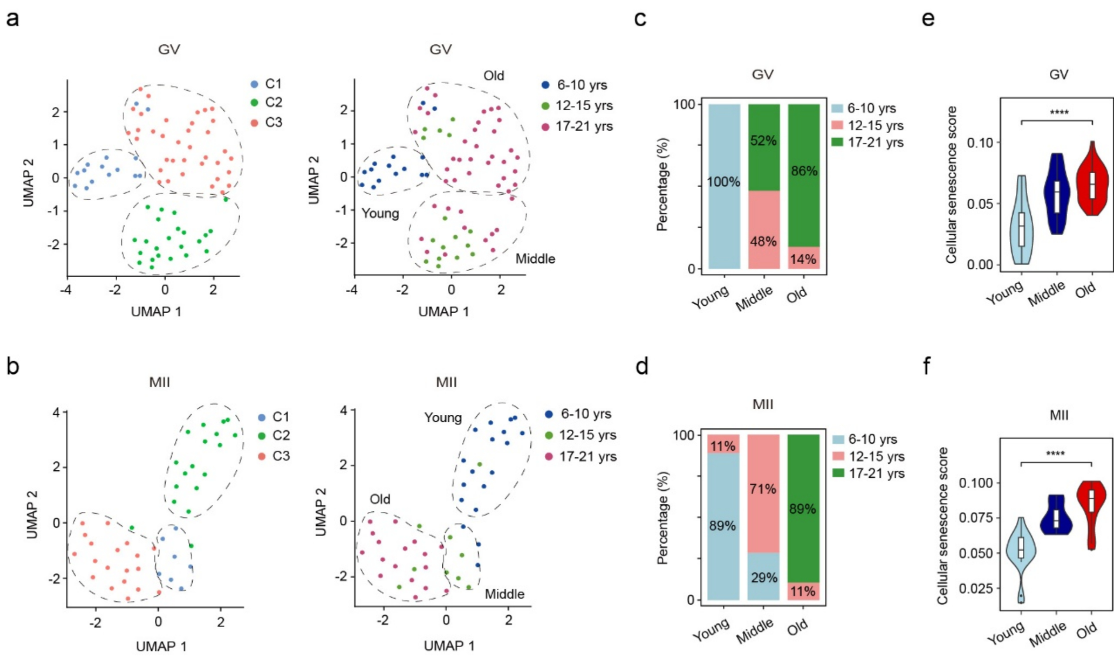
Extended Data Fig. 1. Quality control of scRNA-seq and scATAC-seq data.

(**a**) The numbers of genes expressed in scRNA-seq data. GV, Germinal Vesicle Oocyte; MII, Metaphase II oocyte; 4C, 4C embryo; 8C, 8C embryo; Mor, morula embryo.

(**b**) The proportions of mitochondrial gene expression in scRNA-seq data.

(**c**) Quality control of scATAC-seq data from 4C and 8C embryos. The x-axis shows the fragment count. The y-axis shows signal-to-noise ratio.

(**d**) Quality control of scATAC-seq data from morula embryos. The x-axis shows the fragment count. The y-axis shows signal-to-noise ratio.



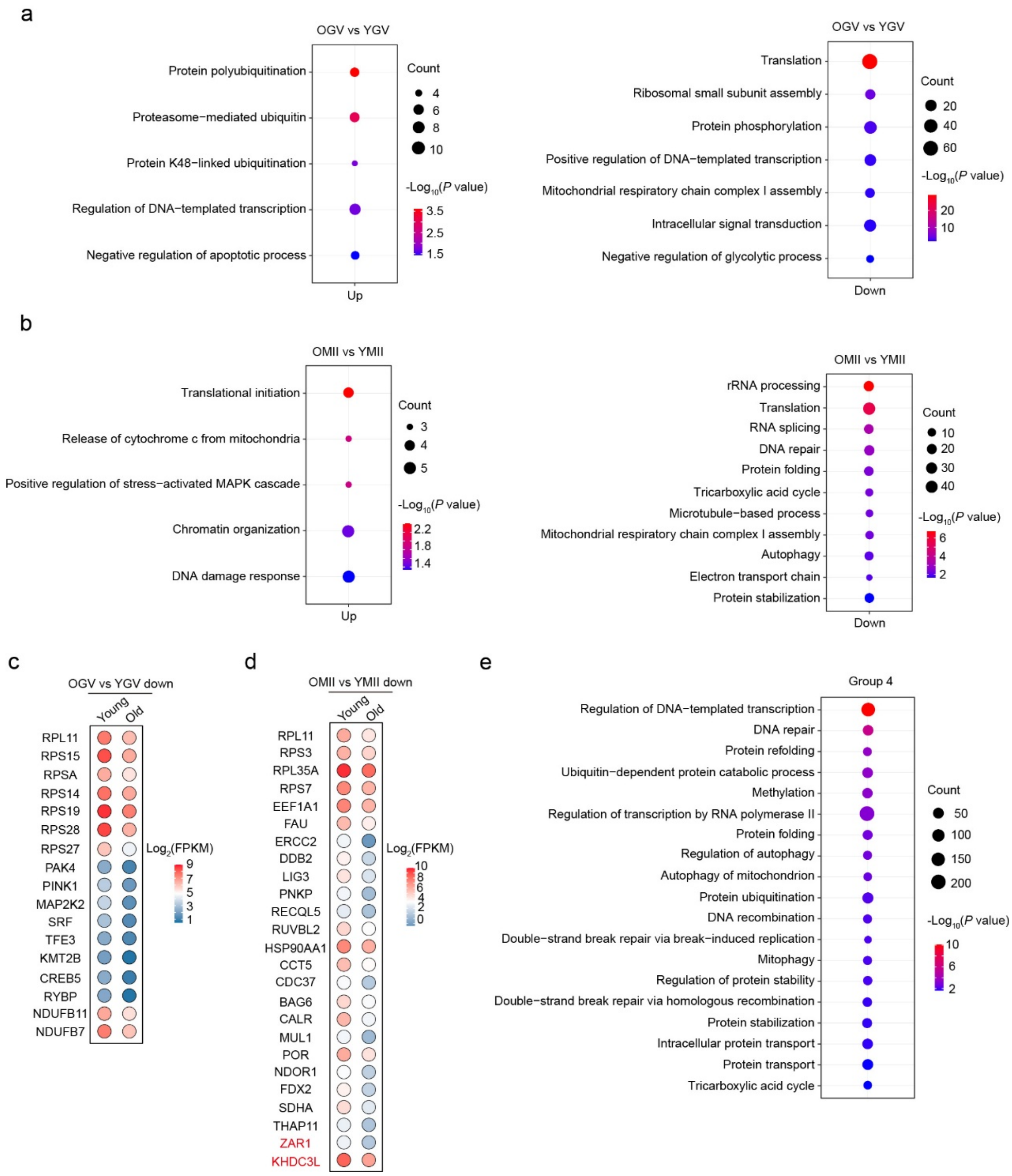
Extended Data Fig. 2. Classification of single GV and MII oocytes by UMAP-based analysis.

(**a, b**) UMAP analysis identified three clusters of single GV (a) and MII (b) oocytes, representing young, middle-aged and old samples.

(**c, d**) Cell compositions of each cluster for GV (c) and MII (d) oocytes.

(**e, f**) Gene set score analysis of cellular senescence genes showed that old cluster had significantly higher senescence score than young cluster for GV (e) and MII (f) oocytes.

Data were analyzed by one-way ANOVA with Tukey's test (e) or the Kruskal-Wallis test followed by Dunn's test (f). ****$P < 0.0001$.



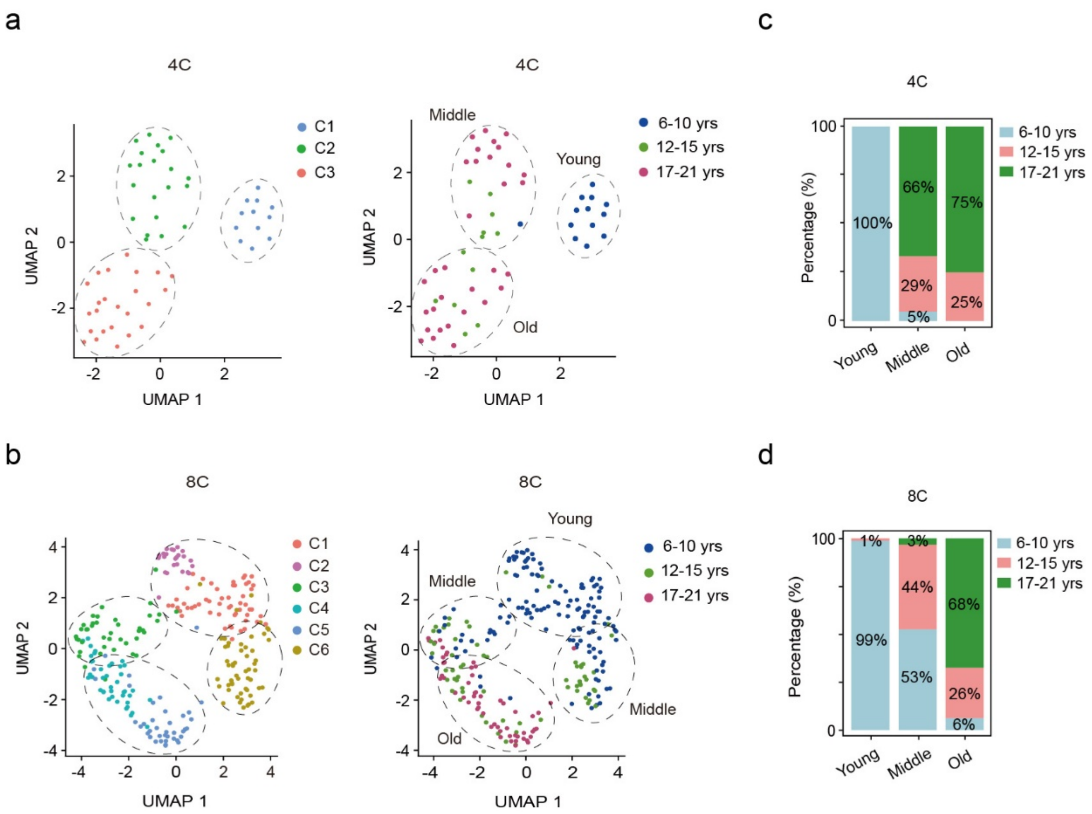
Extended Data Fig. 3. Impairment of maternal mRNA expressions and dynamics during meiosis in old oocytes.

(**a**) Top biological processes enriched for up-regulated (left panel) and down-regulated (right panel) DEGs in old GV oocytes compared to young GV counterparts.

(**b**) Top biological processes enriched for up-regulated (left panel) and down-regulated (right panel) DEGs in old MII eggs compared to young MII counterparts.

(**c, d**) Example genes showing down-regulation in old GV (c) or MII (d) oocytes compared to young counterparts.

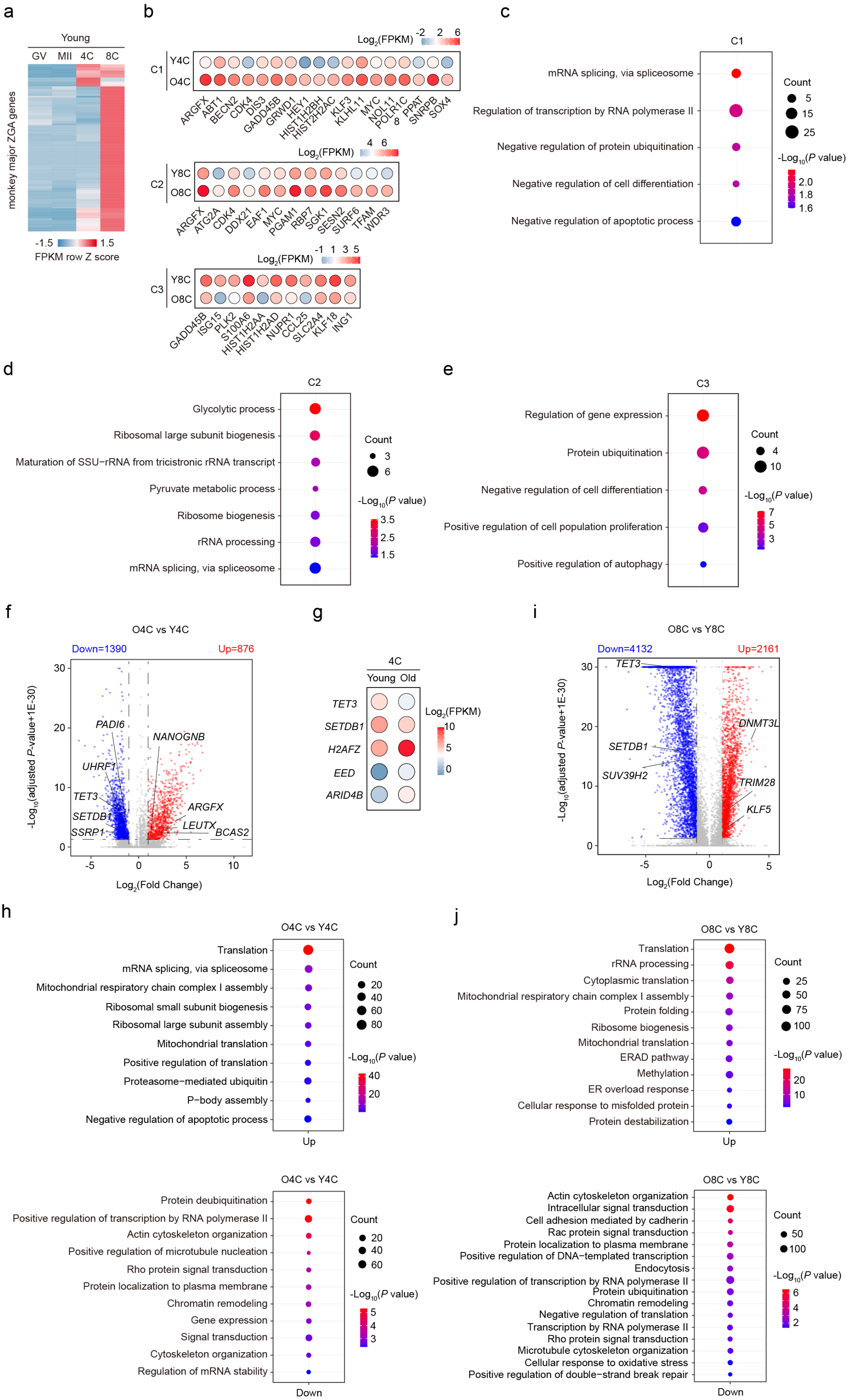
(**e**) Top biological processes enriched for 2806 genes (group 4) showing premature degradation during GV-MII transition in old oocytes.



Extended Data Fig. 4. Classification of single 4C and 8C blastomeres by UMAP-based analysis.

(**a, b**) UMAP analysis identified three clusters of single 4C (a) and 8C (b) blastomeres, representing young, middle-aged and old samples.

(**c, d**) Cell composition of each cluster for 4C (c) and 8C (d) blastomeres.



a
Young
GV MII 4C 8C
monkey major ZGA genes
-1.5 1.5
FPKM row Z score
b
Log2(FPKM) -2 2 6
C1 Y4C O4C
ARGFX ABT1 BECN2 CDK4 DIS3 GADD45B GRWD1 HEY1 HIST1H2BH HIST2H2AC KLF3 KLHL11 MYC NOL11 POLR1C 8 PPAT SNRPB SOX4
Log2(FPKM) 4 6
C2 Y8C O8C
ARGFX ATG2A CDK4 DDX21 EAF1 MYC PGAM1 RBP7 SGK1 SESN2 SURF6 TFAM WDR3
Log2(FPKM) -1 1 3 5
C3 Y8C O8C
GADD45B ISG15 PLK2 S100A6 HIST1H2AA HIST1H2AD NUPR1 CCL25 SLC2A4 KLF18 ING1
c
C1
mRNA splicing, via spliceosome
Regulation of transcription by RNA polymerase II
Negative regulation of protein ubiquitination
Negative regulation of cell differentiation
Negative regulation of apoptotic process
Count 5 15 25
-Log10(P value) 2.0 1.8 1.6
d
C2
Glycolytic process
Ribosomal large subunit biogenesis
Maturation of SSU−rRNA from tricistronic rRNA transcript
Pyruvate metabolic process
Ribosome biogenesis
rRNA processing
mRNA splicing, via spliceosome
Count 3 6
-Log10(P value) 3.5 2.5 1.5
e
C3
Regulation of gene expression
Protein ubiquitination
Negative regulation of cell differentiation
Positive regulation of cell population proliferation
Positive regulation of autophagy
Count 4 10
-Log10(P value) 7 5 3
f
O4C vs Y4C
Down=1390 Up=876
PADI6 UHRF1 TET3 SETDB1 SSRP1 NANOGNB ARGFX LEUTX BCAS2
-Log10(adjusted P-value+1E-30)
Log2(Fold Change)
g
4C
Young Old
TET3 SETDB1 H2AFZ EED ARID4B
Log2(FPKM) 10 5 0
i
O8C vs Y8C
Down=4132 Up=2161
TET3 SETDB1 SUV39H2 DNMT3L TRIM28 KLF5
-Log10(adjusted P-value+1E-30)
Log2(Fold Change)
h
O4C vs Y4C
Translation
mRNA splicing, via spliceosome
Mitochondrial respiratory chain complex I assembly
Ribosomal small subunit biogenesis
Ribosomal large subunit assembly
Mitochondrial translation
Positive regulation of translation
Proteasome−mediated ubiquitin
P−body assembly
Negative regulation of apoptotic process
Count 20 40 60 80
-Log10(P value) 40 20
Up
O4C vs Y4C
Protein deubiquitination
Positive regulation of transcription by RNA polymerase II
Actin cytoskeleton organization
Positive regulation of microtubule nucleation
Rho protein signal transduction
Protein localization to plasma membrane
Chromatin remodeling
Gene expression
Signal transduction
Cytoskeleton organization
Regulation of mRNA stability
Count 20 40 60
-Log10(P value) 5 4 3
Down
j
O8C vs Y8C
Translation
rRNA processing
Cytoplasmic translation
Mitochondrial respiratory chain complex I assembly
Protein folding
Ribosome biogenesis
Mitochondrial translation
ERAD pathway
Methylation
ER overload response
Cellular response to misfolded protein
Protein destabilization
Count 25 50 75 100
-Log10(P value) 20 10
Up
O8C vs Y8C
Actin cytoskeleton organization
Intracellular signal transduction
Cell adhesion mediated by cadherin
Rac protein signal transduction
Protein localization to plasma membrane
Positive regulation of DNA−templated transcription
Endocytosis
Positive regulation of transcription by RNA polymerase II
Protein ubiquitination
Chromatin remodeling
Negative regulation of translation
Transcription by RNA polymerase II
Rho protein signal transduction
Microtubule cytoskeleton organization
Cellular response to oxidative stress
Positive regulation of double−strand break repair
Count 50 100
-Log10(P value) 6 4 2
Down

Extended Data Fig. 5. Precocious and excessive ZGA genes expression in aged monkey 4C and 8C embryos.

(**a**) Heatmap showing the relative expression of 874 major ZGA genes in young monkey embryos.

(**b**) Relative expression of C1 ZGA genes (precocious expression in O4C), C2 ZGA genes (excessive expression in O8C), and C3 ZGA genes (reduced expression in O8C).

(**c-e**) Top biological processes enriched for C1 (c), C2 (d), and C3 (e) ZGA genes.

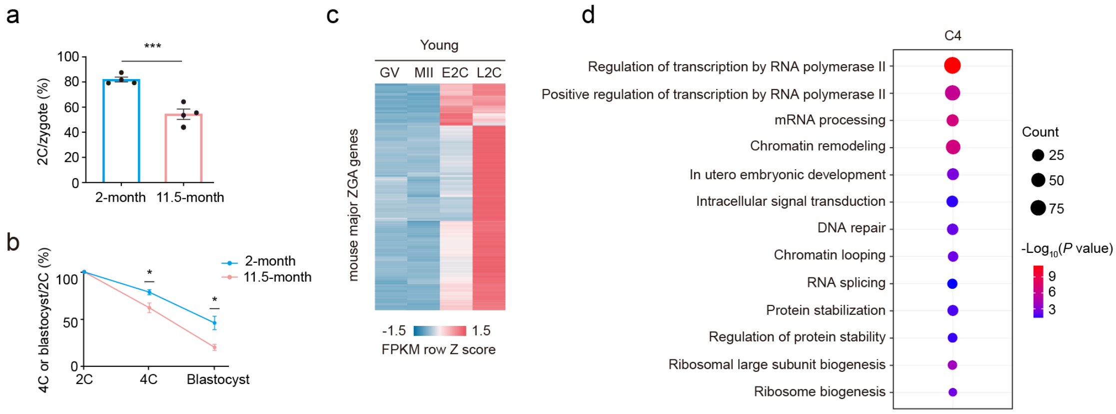
(**f**) Volcano plots showing up-regulated and down-regulated DEGs in old 4C (O4C) embryos relative to young 4C (Y4C) counterparts.

(**g**) Relative expression of some epigenetic genes in O4C and Y4C embryos.

(**h**) Top biological processes enriched for up-regulated DEGs (upper panel) and down-regulated DEGs (lower panel) in O4C compared to Y4C embryos.

(**i**) Volcano plots showing up-regulated and down-regulated DEGs in old 8C (O8C) embryos compared to young 8C (Y8C) counterparts.

(**j**) Top biological processes enriched for up-regulated DEGs (upper panel) and down-regulated DEGs (lower panel) in O8C compared to Y8C embryos.



Extended Data Fig. 6. Precocious and excessive ZGA is not conserved in aged mouse embryos

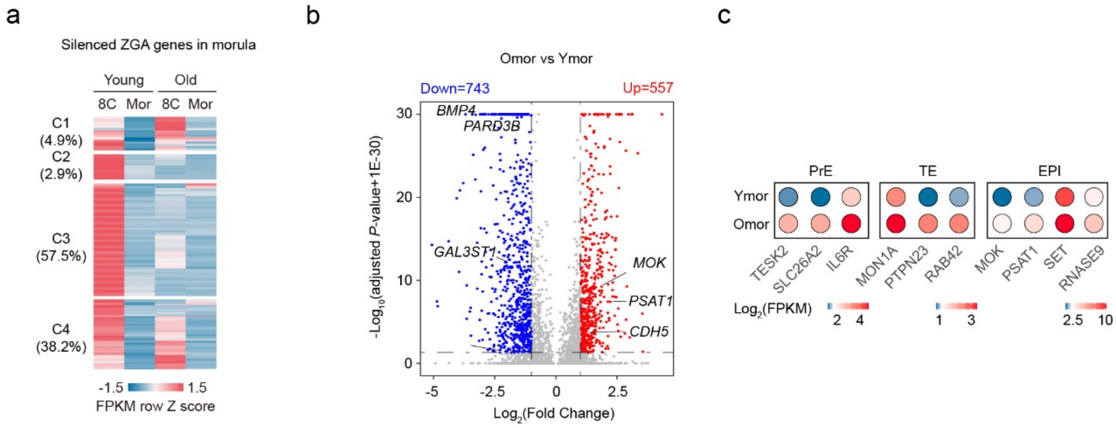
(**a**) Percentages of zygotes progressing to 2C after fertilization by ICSI. MII eggs were collected from 2-month old and 11.5-month old females.

(**b**) Percentages of 2C embryos progressing to 4C and blastocyst stages in young and old mice.

(**c**) Heatmap showing the relative expression of 1274 major ZGA genes in young mouse embryos.

(**d**) Top biological processes enriched for the majority (79.7%) of mouse ZGA genes (C4) not affected by reproductive aging.

Data in (a, b) were expressed as mean ± SEM and were analyzed by a two-tailed Student's *t*-test. *$P < 0.05$, ***$P < 0.001$.

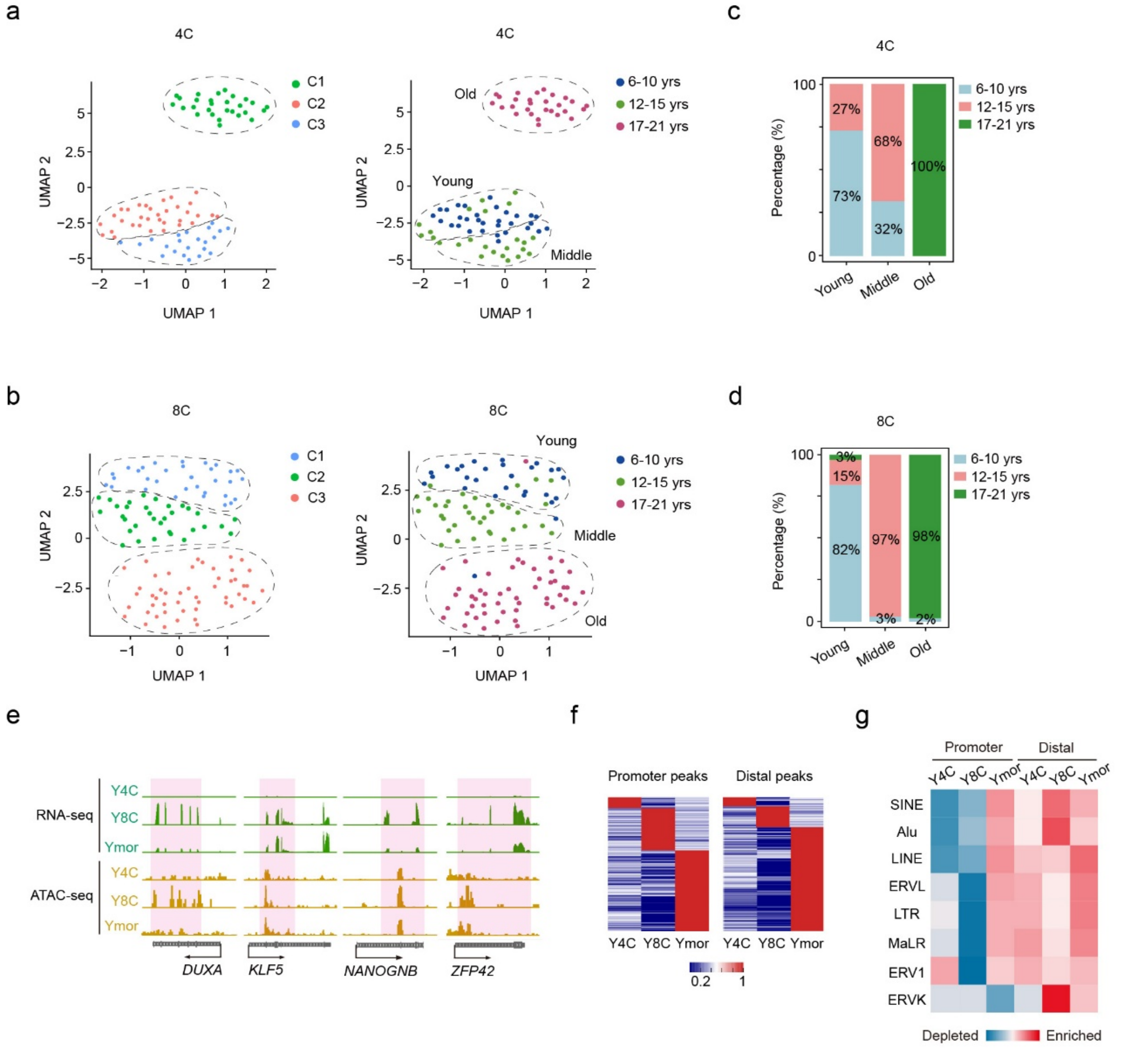


Extended Data Fig. 7. Expression of morula-stage genes in aged monkey embryos.

(**a**) Heatmap showing the aberrant expression of 306 ZGA genes which are subject to silencing in young morula. 15 ZGA genes (4.9%) in C1 showed compromised silencing in old morula.

(**b**) Volcano plots showing the up-regulated and down-regulated DEGs in old morulae (Omor) relative to young morulae (Ymor).

(**c**) Compared to young morulae, old morulae expressed higher level of lineage genes (trophectoderm, TE: *MON1A*, *PTPN23*, *RAB42*; epiblast, EPI: *MOK*, *PSAT1*, *SET*, *RNASE9*; primitive endoderm, PrE: *TESK2*, *SLC26A2*, *IL6R*).



Extended Data Fig. 8. Chromatin accessibility analyses in monkey embryos.

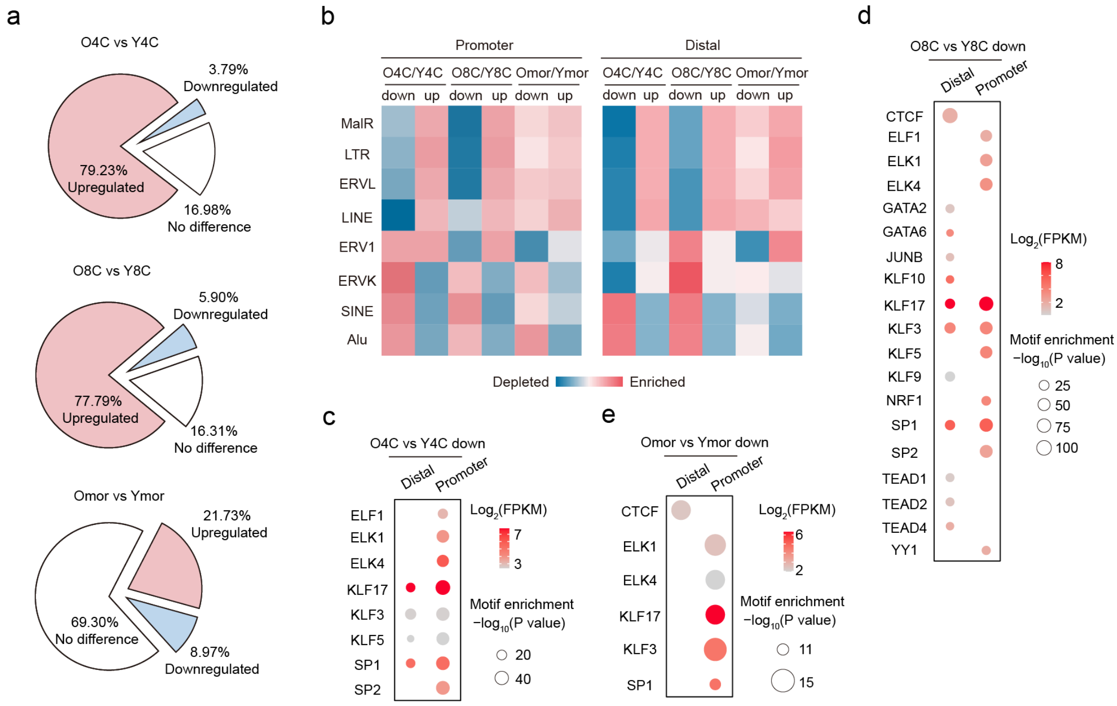
(**a, b**) Using scATAC-seq data, UMAP analysis grouped 4C (a) and 8C (b) blastomeres into three clusters, representing young, middle-aged and old samples.

(**c, d**) Cell composition of each cluster for 4C (c) and 8C (d) blastomeres.

(**e**) Several representative ZGA genes (*DUXA*, *KLF5*, *NANOGNG*, *ZFP42*) showed concordant ATAC-seq and RNA-seq signals, indicating the high quality of scATAC-seq data.

(**f**) Stage-prevalent ATAC-seq peaks at promoters (± 2500 bp from TSS) and nearby distal regions in young embryos.

(**g**) Transposable elements (TEs) resided in promoters and distal peaks of young 4C, 8C, and morula embryos.

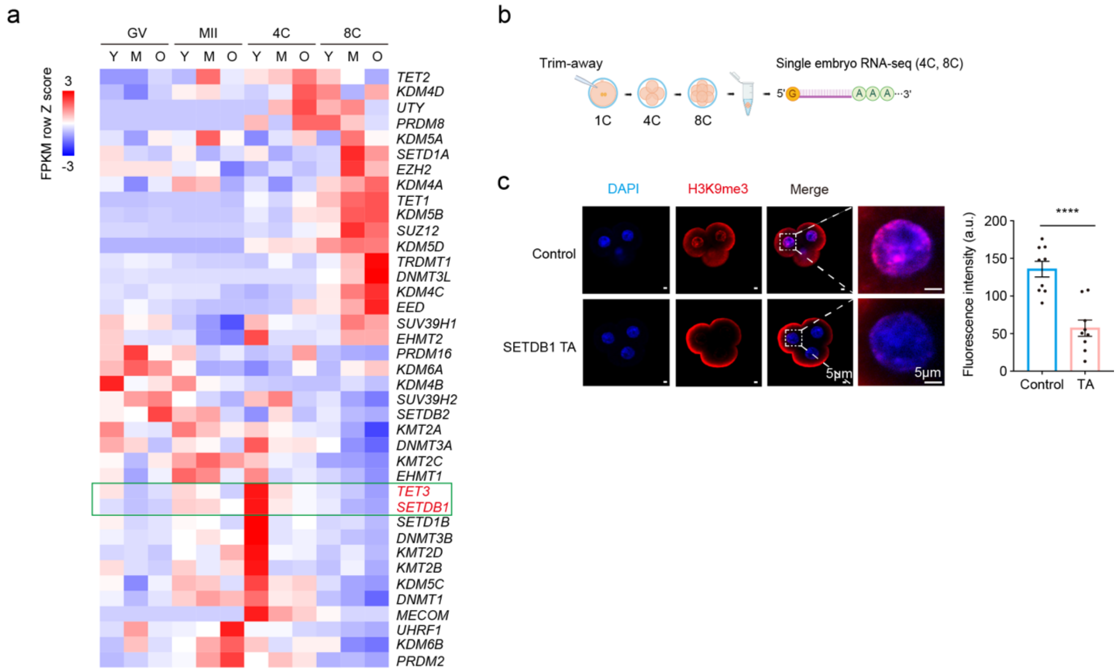


Extended Data Fig. 9. Precocious and enhanced chromatin accessibility in aged monkey embryos.

(**a**) Comparison of overall chromatin accessibility between old and young embryos at 4C, 8C, and morula stage.

(**b**) Transposable elements (TEs) resided in promoters and distal regions showing increased or decreased chromatin accessibility in old embryos relative to young counterparts.

(**c-e**) Potential binding TFs at promoters and distal regions showing decreased accessibility in old embryos compared to young counterparts at 4C (c), 8C (d) and morula (e) stages.



Extended Data Fig. 10. Knock down maternal SETDB1 in monkey embryos via trim-away.

(**a**) Heatmap showing the relative expression levels (FPKM) of epigenetic regulators involved in methylation of DNA, H3K27, H3K9, and H3K4 in GV to 8C embryos from different age groups.

(**b**) Schematic showing trim-away (TA) knockdown strategy and sample collection in monkey embryos.

(**c**) TA efficiency was tested in mouse embryos. Immunostaining showed the significantly reduced H3K9me3 level.

Data in (c) were expressed as mean ± SEM and were analyzed by a two-tailed Student's *t*-test. $****P < 0.0001$.

Supplementary Table 1. Information of the monkey samples for scRNA-seq and scATAC-seq.

Samples of scRNA-seq

Stage	No. of single cells
GV	74
MII	44
4C	57
8C	253
Morula	135
Total	563

Samples of scATAC-seq

Stage	No. of single cells
4C	83
8C	116
Morula	217
Total	416

Supplementary Table 2. Information of the mouse samples for RNA-seq.

Stage	Total No. of samples	No. of samples from 2-month-old mice	No. of samples from 11.5-month-old mice
GV	3	3	0
MII	3	3	0
E2C	14	8	6
L2C	14	8	6

Note: Each oocyte sample contains 9-12 oocytes; each embryo sample contains one embryo.

Supplementary Table 3. Information of scRNA-seq samples from morula stage embryos.

Grouping by embryo donor age	No. of single cells
Young (6-10 years old)	72
Old (≥17 years old)	63
Total	135

Supplementary Table 4. Information of scATAC-seq samples from morula stage embryos.

Grouping by embryo donor age	No. of single cells
Young (6-10 years old)	156
Old (≥17 years old)	61
Total	217

Supplementary Table 5. Primers for qRT-PCR.

Name	Sequences
DUXA-F	5’-CCCAGGTTACGCTACCAAACA-3’
DUXA-R	5’-CCAGGTTGATCTTGCCCCTG-3’
KDM5B-F	5’-AAGAGTAGCATCAAGCAAGA-3’
KDM5B-R	5’-CACATAAGAGACAGACATACAG-3’
KLF5-F	5’-TGCATCTGTCTCTGTGATTACCA-3’
KLF5-R	5’-AAATGTCTAGGCTCCGCGTC-3’
POU5F1-F	5’-GAGAACCGAGTGAGAGGCAG-3’
POU5F1-R	5’-CACACTCGGACCACATCCTT-3’
DPPA2-F	5’-TACCACTCGCAGGAGGATGA-3’
DPPA2-R	5’-TAGCACAGTCGGGGCATAAC-3’
GATA6-F	5’-GTGCCCAGACCACTTGCTAT-3’
GATA6-R	5’-CCCTGAGGCTGTAGGTTGTG-3’
EED-F	5’-GGCAAGATGGAAGATGATATAG-3’
EED-R	5’-CGAATAGCAGCACCACAT-3’
DNMT3L-F	5’-CCATAAGGAGCAGCAGGCAG-3’
DNMT3L-R	5’-GGGGGAGAAAGCAGTTCTTCA-3’
RFPL4A-F	5’-CCAGCAGTATGCCTATGACCCC-3’
RFPL4A-R	5’-ACTGGCTGGATTGATCAGAGGA-3’
SETDB1-F	5’-AGATGTCTGGAGAACTAAGC-3’
SETDB1-R	5’-TGTCTGGATGGCAATAAGG-3’
TET3-F	5’-CAAGACACCTCGCAAGTT-3’
TET3-R	5’-CGGCAGTCAATCGCTATT-3’
GAPDH-F	5’-GAGAACGGGAAGCTCGTCAT-3’
GAPDH-R	5’-TCGCCCCACTTGATTTTGGA-3’