

1 **Figure S1. Pluripotent stem cells derived from mouse spermatogonial stem cells**
2 **and sample information of RNA-seq Data, related to Figure 1**

3 (A) Immunofluorescence of ZBTB16 (red, top) co-stained with OCT4 (green, top), and
4 GFRA1 (red, bottom) co-stained with DDX4 (pink, bottom) in mSSCs (SSC0606 p10).
5 Scale bar, 20 μm .

6 (B) Immunofluorescence of DDX4 (red) and PNA (green) in the testicular paraffin
7 sections from *Kit*^{w/wv} mice transplanted with (top) or without (bottom) mSSCs
8 (SSC0606 p10), respectively. Scale bar, 40 μm .

9 (C) Immunofluorescence of OCT4, NANOG, SSEA1 and DDX4 (red) in gPSCs
10 (gPSC-1 p7). Scale bar, 20 μm .

11 (D) Metaphase spread of karyotype showing 40 chromosomes of gPSC-1 (p10). Scale
12 bar, 20 μm .

13 (E) H&E staining of paraffin sections of teratoma, which were formed after
14 subcutaneous injection of gPSC-1 (p15) into nude mice. Endodermal gland structure of
15 gastrointestinal (left); mesodermal muscle fibre (middle); ectodermal cornified layer
16 and keratinocyte (right). Scale bar, 100 μm .

17 (F) Bright field and fluorescence images showing E12.5 embryos of KM mouse and
18 chimaera embryo produced by blastocyst injection of CAG-EGFP gPSC-2 (p10), 4 out
19 of 7 embryos were chimaeras after gPSCs injection. Scale bar, 2 mm.

20 (G) Bright field and fluorescence images of the E12.5 genital ridges derived from
21 blastocyst injection of *Oct4*-EGFP gPSC-1 (p10), 5 out of 13 pairs of genital ridges
22 were GFP positive after gPSCs injection. Scale bar, 500 μm .

23 (H) Chimaeras produced from B6D2F1 (black coat color) gPSC-1.

24 (I) Summary of chimeric assays of diploid blastocyst injection using gPSC-1.

25 (J) Sample information of reprogramming cells (original condition), gPSCs, and ESCs
26 for scRNA-seq analyzed in this study.

27 (K) UMAP plot showing reprogramming cells from two batches of SSC
28 reprogramming, gPSCs and ESCs.

- 1 (L) Boxplot showing the numbers of genes (top) and UMIs (bottom) detected in each
- 2 single cell.
- 3 (M) UMAP plot showing reprogramming cells from day 0 to day 27.
- 4 (N) Violin plots showing the expression levels ($\log(\text{TPM}/10+1)$) of specific genes in
- 5 each cluster.
- 6

1 **Figure S2. Transcriptomic signatures of SSC reprogramming, related to Figure 1**

2 (A) UMAP plot showing the RNA velocity analysis results of SSC reprogramming,
3 each arrow represents the predicted developmental direction of the individual cell.

4 (B) Cell trajectory of the early stage during SSC reprogramming inferred by Monocle2.
5 Arrows indicate the developmental orders of these cells.

6 (C) PAGA results visualized by the temporal information of each cluster during the
7 early stage of reprogramming.

8 (D) PAGA results visualized by the scores of distinct signatures. The color key from
9 dark blue to bright yellow indicates low to high signature scores, respectively.

10 (E) UMAP plot showing the transcriptome data of the successful and failed branches
11 of SSC reprogramming.

12 (F) Enriched GO terms of four representative clusters of failed branches.

13 (G) Violin plots showing the expression dynamics of KEGG pathway
14 ‘Glycolysis/Gluconeogenesis’ (mmu00010) in the failed branches of SSC
15 reprogramming.

16 (H) Pearson correlation between signature score of pluripotency-related genes and
17 glycolysis- as well as DNA transmethylase & demethylase-related genes based on gene
18 expression in each single cell amongst RPG1/2/3/4. Genes sets used in each signature
19 could be found in Table S2.

20 (I) Venn diagram showing DEGs in the stage 1 and stage 2 of three reprogramming
21 processes, corresponding to Figure 1M.

22 (J) Bubble plots showing GO terms down-regulated in stage 1 (top) and stage 2 (bottom)
23 of three reprogramming processes, corresponding to Figure 1M.

24

1 **Figure S3. The Developmental Potential of PSCs Estimated by Tetraploid**
2 **Complementation, the Methylation Level of SSCs and PSCs, related to Figure 3**

3 (A) E12.5 embryos (left) produced by tetraploid complementation (4N-comp) of ESCs,
4 5C-gPSC-S2, and gPSC-S2, respectively, and their corresponding E12.5 genital ridges
5 (middle and right). Scale bar, 2 mm (left), 500 μ m (middle and right).

6 (B) ESC (top) and gPSC-S2 (bottom) 4N-comp pups and their corresponding placentae.
7 Note, * Pups survived beyond 4 days postnatal; # Pups died within 4 days postnatal.

8 (C) Bright field, fluorescence images of E3.5 *Oct4*-EGFP blastocysts flushed from an
9 ICR female mouse mated with F1-5 ESC (top), 5C-gPSC-S2 (middle), and gPSC-S2
10 (bottom) 4N-comp mice, respectively. Scale bar, 100 μ m.

11 (D) An 11-week-old F1-5 ESC 4N-comp mouse with a uniformly black coat and its F₁
12 progeny from its mating to an ICR female mouse.

13 (E) A 16-week-old gPSC-S2 4N-comp mouse with a uniformly black coat and its F₁
14 progeny from its mating to an ICR female mouse.

15 (F) The global DNA methylation levels of SSCs (p20), gPSCs (p25), 5C-gPSCs (p25),
16 and ESCs (p10) around the CGI regions and gene bodies of indicated samples. Samples
17 are indicated by colors.

1 **Figure S4. Sample information and quality control of single cell RNA-sequencing**
2 **data of 5C induced SSC reprogramming, related to Figure 4**

3 (A) Sample information of DMSO or 5C induced reprogramming cells for scTrio-seq2
4 (transcriptome) analysis in this study.

5 (B) Boxplot showing the numbers of genes (top) and UMIs (bottom) detected in each
6 single cell collected at different time points, corresponding to Figure S4A.

7 (C) UMAP plots showing the integration of transcriptome data from the original
8 condition (control), DMSO and 5C induced reprogramming cells, cells are colored by
9 clusters (left and middle) and batches (right).

10 (D) UMAP plot showing the integration of transcriptome data from the successful
11 branch of SSC reprogramming (DMSO and 5C groups), mouse *in vivo* germ cells and
12 gonadal somatic cells from E6.5 to PND5.5. Orange dashed arrow indicates the
13 trajectory of *in vivo* germ cell development, red dashed arrow indicates the trajectory
14 of SSC reprogramming. Cells are colored by batches.

15 (E) UMAP plots showing the integration of transcriptome data from the successful
16 branch of SSC reprogramming (control group), mouse *in vivo* germ cells and gonadal
17 somatic cells from E6.5 to PND5.5. Grey dots indicate *in vivo* germ cells or gonadal
18 somatic cells, colored dots with black outline indicate reprogramming cells or ESCs
19 (left). Cells are colored by clusters (left) and batches (right). Orange dashed arrow
20 indicates the trajectory of *in vivo* germ cell development, green dashed arrow indicates
21 the trajectory of SSC reprogramming.

22 (F) Scmap projection of the successful branch between SSC reprogramming (control
23 group) and mouse *in vivo* germ cells. The color key from bright yellow to dark blue
24 indicates low to high ratio of cells projected.

25 (G) Violin plots showing the expression levels of stage-specific genes during *in vivo*
26 germ cell development.

27 (H) Immunofluorescence of *Oct4*-EGFP (green) co-stained with DPPA3 (pink) and
28 TFAP2C (red) in domed colonies during SSC reprogramming. Scale bar, 50 μ m.

1 **Figure S5. Identification of gene editing in SSC reprogramming, related to Figure**

2 **5**

3 (A) SCENIC TF-targets network of representative regulons highlighted in Figure 5B,
4 target genes related GO terms in Figure 5B were labeled.

5 (B) q-PCR analysis of the relative expression of *Rhox5*, *Otx2*, and *Prdm14* normalized
6 to the geometric mean of *Rps2* and *Gapdh* of CRISPR-CasRx transfected gPSCs.
7 Unpaired two-tailed Student's *t*-test, **P* < 0.05, ***P* < 0.01.

8 (C) Relative expression of RHOX5 (top), PRDM14 (middle), and OTX2 (bottom)
9 measured by western blotting of sgNC-, sg*Rhox5/Prdm14/Otx2*-transfected gPSCs.

10 (D) Bright field images of SSCs transfected with CRISPR-CasRx for targeting empty
11 vector and *Rhox5*, respectively. Scale bar, 200 μ m.

12 (E-G) Immunofluorescence of ZBTB16 (top, red) co-stained with OCT4 (top, green),
13 GFRA1 (bottom, red) co-stained with DDX4 (bottom, pink) in sg*Rhox5* 2#-, sg*Prdm14*
14 2#-, and sg*Otx2* 2#-transfected SSCs. Scale bars, 20 μ m.

15 (H) Bar plots showing the number of OG(+) colonies reprogrammed from sgNC-,
16 sg*Prdm14*-, and sg*Otx2* transfected SSCs in 5C condition on day 19. Unpaired two-
17 tailed Student's *t*-test, ***P* < 0.01.

18 (I) (Top) Immunofluorescence of *Oct4*-EGFP (green) co-stained with RHOX5 (i, red)
19 and DPPA3 (ii, pink) in OG(+) colonies reprogrammed from sgNC- and sg*Rhox5* 2#-
20 transfected SSCs. Scale bars, 50 μ m; (bottom) Dot plots showing the relative intensity
21 of RHOX5 and DPPA3 in OG(+) colonies corresponding to (i) and (ii), respectively.
22 Unpaired two-tailed Student's *t*-test, *****P* < 0.0001.

23

1 **Figure S6. Quality control of single cell methylome data and methylation signatures at**
2 **specific elements and regions during SSC reprogramming, related to Figure 6**

3 (A) Sample information of DMSO or 5C induced reprogramming cells for scTrio-seq2 (DNA
4 methylome) analysis in this study.

5 (B) Boxplots showing the numbers of CpG, 1X CpG depth, mapping ratio and bisulfite
6 conversion rate (BSCR) detected in DMSO (top)- and 5C (bottom)-induced reprogramming
7 cells.

8 (C) The DNA methylation level around the CGI regions (top) and gene bodies (bottom) of all
9 genes in DMSO and 5C induced reprogramming cells.

10 (D) Volcano plots showing the correlation between gene expression and the DNA methylation
11 level of the corresponding promoters in the successful branch of SSC reprogramming. The x
12 axis represents the differential expression (genes with $|\log(\text{fold change})| \geq 1$, adjusted $P <$
13 0.01 were designated as differential expressed genes, DEGs) between comparison groups, and
14 positive values indicate the higher expression in RPG2/3/4. The y axis represents the DNA
15 methylation difference of promoter (genes with absolute methylation difference $> 30\%$ and P
16 < 0.05 were designated as differential methylated promoter genes, DMPGs) between the
17 comparison groups, and negative values indicate the lower methylation in RPG2/3/4. Red dots
18 indicate the DEGs with differentially methylated promoters (DEGs & DMPGs). Blue dots
19 indicate the DEGs without differentially methylated promoters (only DEGs).

20 (E) Pearson correlation coefficient between gene expression and promoter DNA methylation
21 levels during SSC reprogramming process of DEGs & DMPGs in (Figure S6D) and genes in
22 Figure 6B ($n = 119$). Histogram in the top showing the distribution of genes. Genes were
23 ordered by Pearson correlation coefficient.

24 (F) Multidimensional scaling (MDS) analysis on the DNA methylome data of SSC
25 reprogramming cells, SSCs, mouse *in vivo* germ cells and sperm, corresponding to Figure 6C.
26 Clusters are indicated by colors.

27 (G) Unsupervised hierarchical clustering based on the DNA methylome data of SSC
28 reprogramming cells, SSC, mouse *in vivo* germ cells and sperm.

29 (H) Boxplots showing the DNA methylation dynamics of indicated genomic features during
30 DMSO and 5C induced reprogramming processes, respectively.

31 (I) Boxplots showing the DNA methylation dynamics of representative maternal ICRs during
32 DMSO and 5C induced reprogramming processes, respectively. The DNA methylation level
33 of maternal ICRs in sperm was the reference for other samples.

34

1 **Figure S7. DNA methylation dynamics at specific elements and regions during the late**
2 **stage of SSC reprogramming, related to Figure 7**

3 (A) Heatmap showing the Pearson's correlation between RPG1/2/3/4 and gPSCs (p1 and p15)
4 based on transcriptome data.

5 (B) Unsupervised hierarchical clustering of ESCs, RPG1/2/3/4, and different passages of
6 gPSCs and 5C-gPSCs based on DNA methylome data.

7 (C) The DNA methylation level around the CGI regions and gene bodies of all genes in gPSCs,
8 5C-gPSCs, and CiPSCs.

9 (D) Three-dimensional MDS analysis of SSCs, ESCs, gPSCs, 5C-gPSCs and CiPSCs based on
10 DNA methylome data. Clusters are colored by samples.

11 (E) Boxplots showing the DNA methylation level of the indicated genomic features in SSCs,
12 ESCs, different passages of gPSCs, 5C-gPSCs and CiPSCs.

13 (F) Boxplots showing the DNA methylation level of representative paternal ICRs in sperm,
14 SSCs, ESCs, different passages of gPSCs and 5C-gPSCs.

15 (G) Lollipop methylation diagrams showing the DNA methylation level of representative
16 maternal ICR (*Peg3*) and paternal ICR (*H19*) in SSCs, ESCs, p25 gPSCs and 5C-gPSCs by
17 sequencing reads in single CpG site resolution.

18 (H) E12.5 embryos (left) produced by tetraploid complementation (4N-comp) of two CiPSC
19 lines, respectively, and their corresponding E12.5 genital ridges (middle and right). Scale bar,
20 2 mm (left), 500 μ m (middle and right).

21 (I) Boxplots showing the DNA methylation level of representative maternal and paternal ICRs
22 in ESCs and different passages of CiPSCs.

23 (J) Line plot showing the mean methylation levels of *Dlk1-Dio3* region in ESCs (p10), R-1
24 CiPSCs (p5 and p25), K-2 CiPSCs (p5 and p25), respectively. Grey shadows indicate gene
25 regions, the red shadow indicates IG-*Gtl2* ICR region.

26 (K) Box plots showing the mean DNA methylation levels across gene bodies (from
27 transcription start site (TSS) to transcription end site (TES)) and the 50-bp flanking regions of
28 each miRNA encoded locus in *Dlk1-Dio3* region in ESCs and CiPSCs. One dot represents a
29 miRNA encoded locus, the same loci in distinct PSCs are connected by grey lines. Paired two-
30 tailed Student's *t*-test was performed between ESCs and CiPSCs.

31

Figure S1

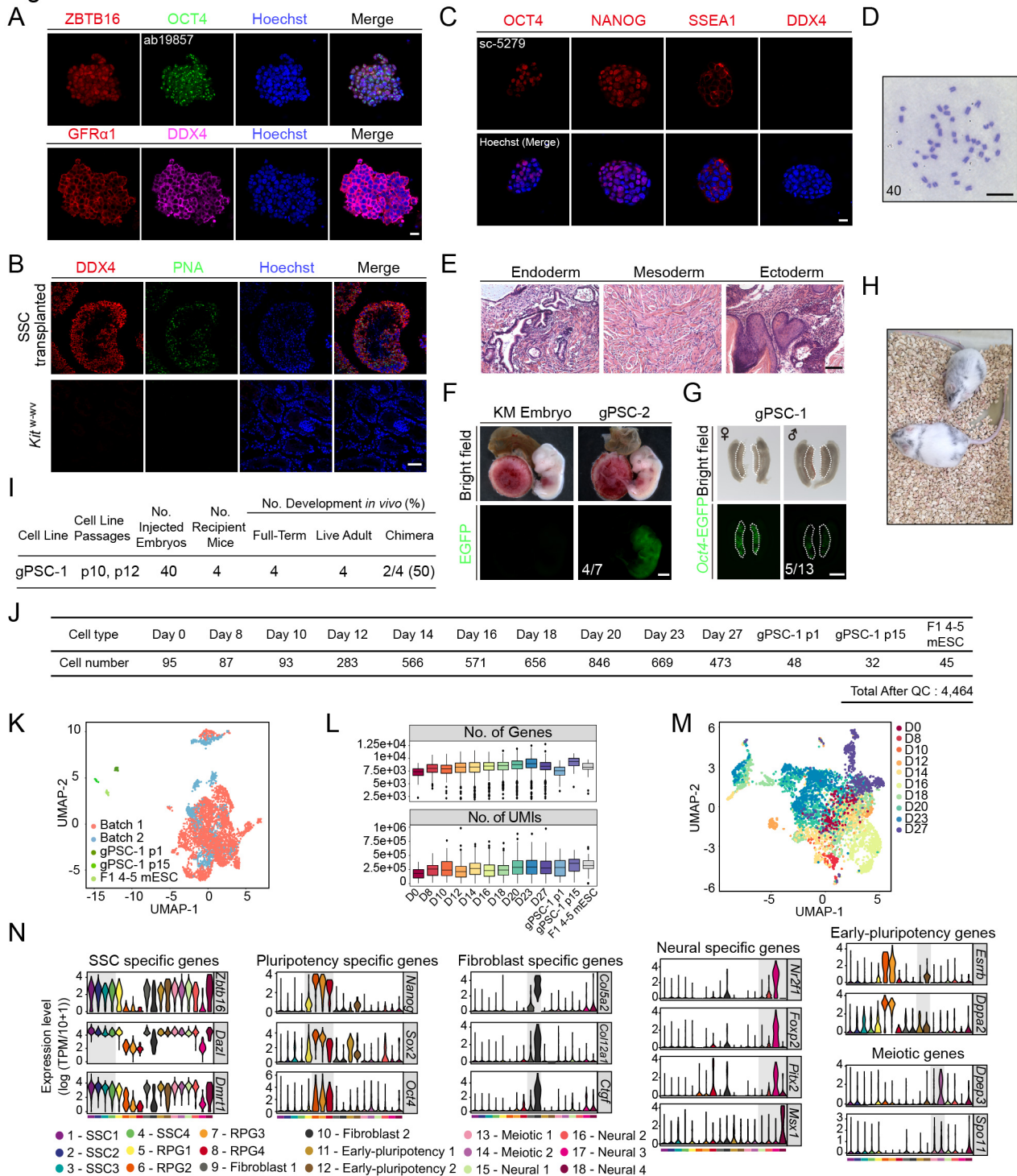


Figure S2

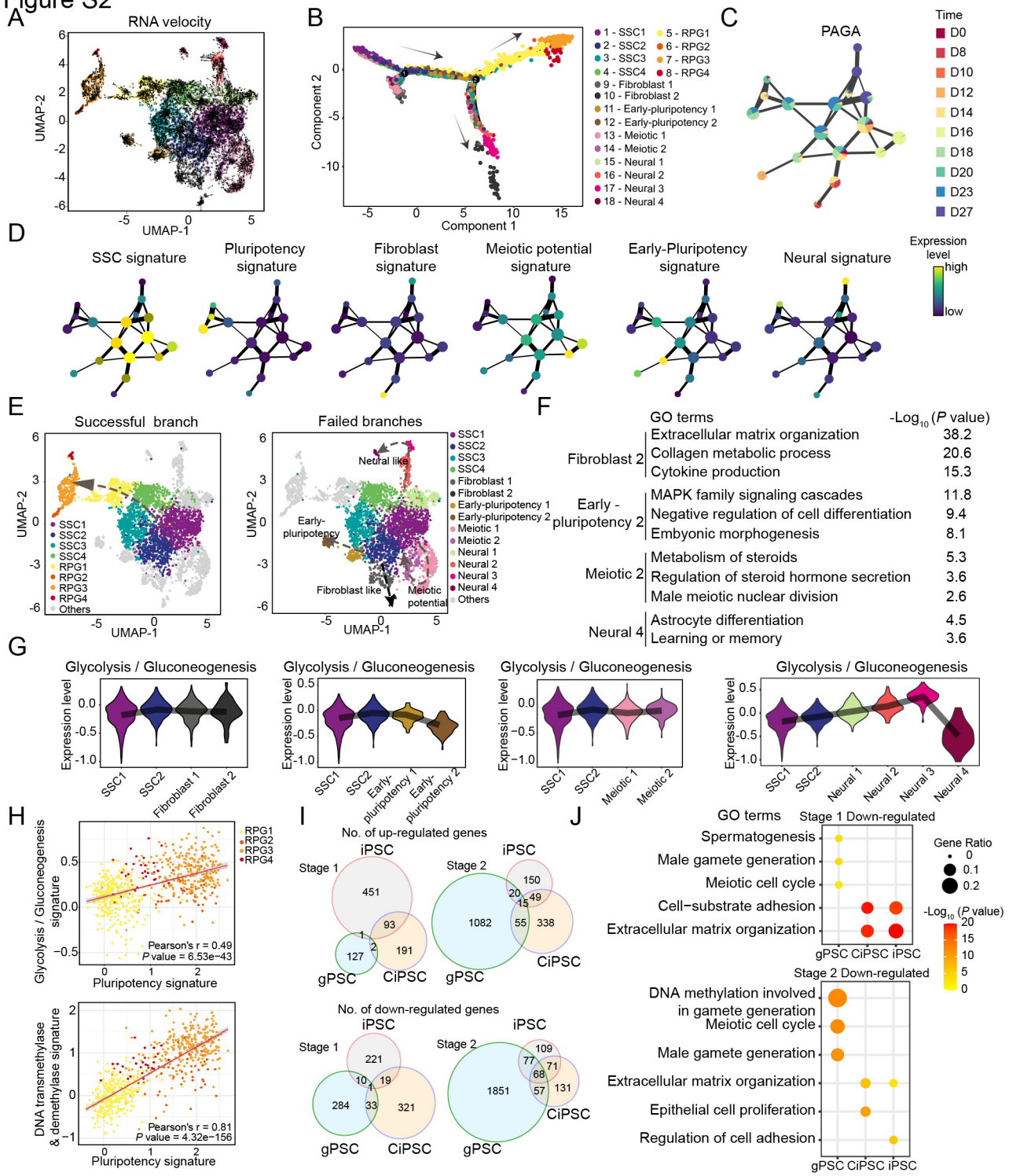


Figure S3

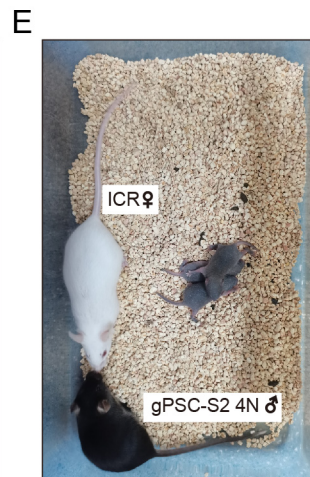
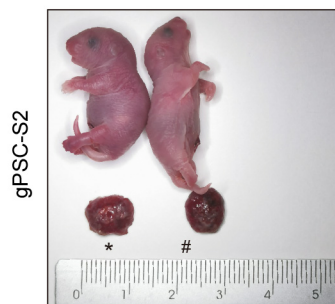
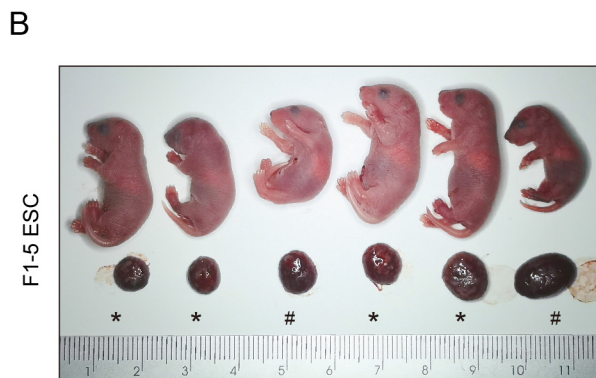
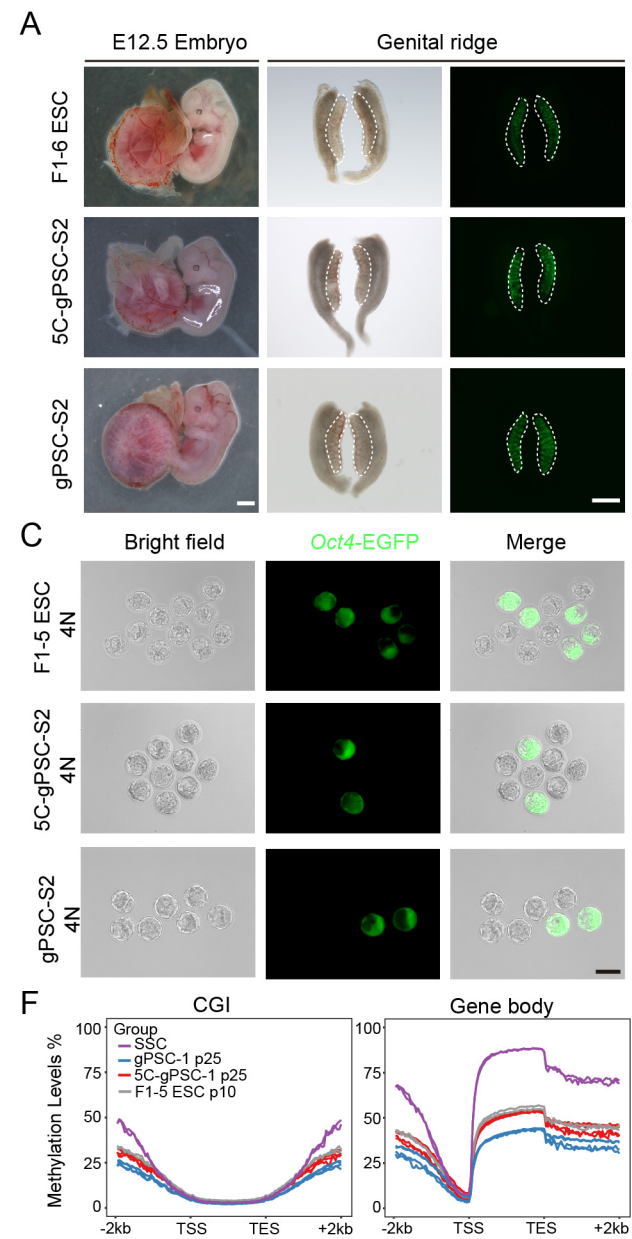


Figure S4

A

DMSO	Day 0	Day 20	Day 24
Cell number	8	266	91

5C	Day 12	Day 14	Day 16
Cell number	368	180	176

Total After QC : 1,089

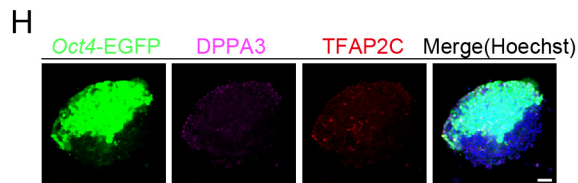
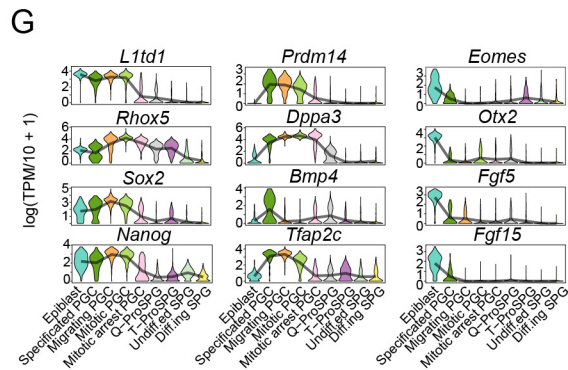
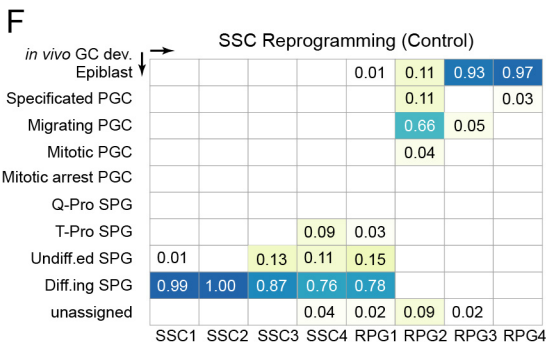
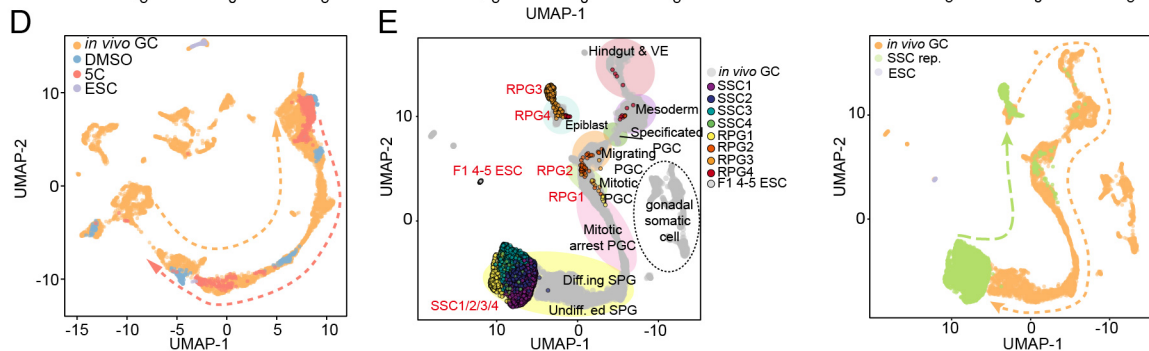
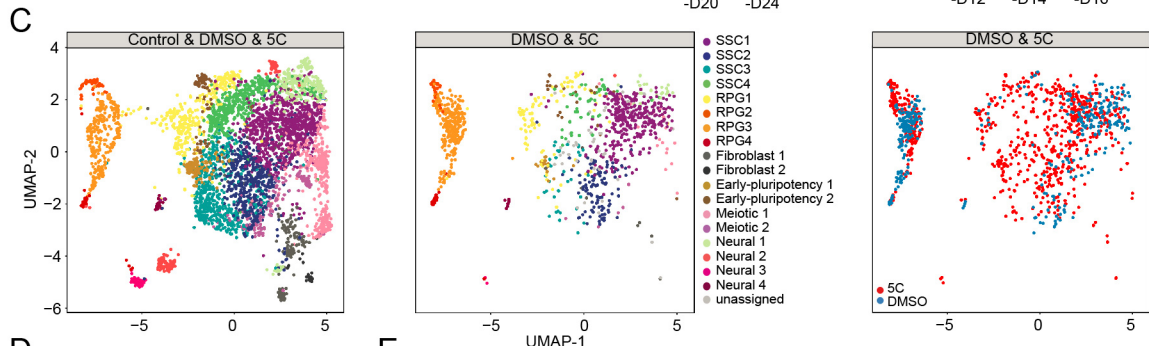
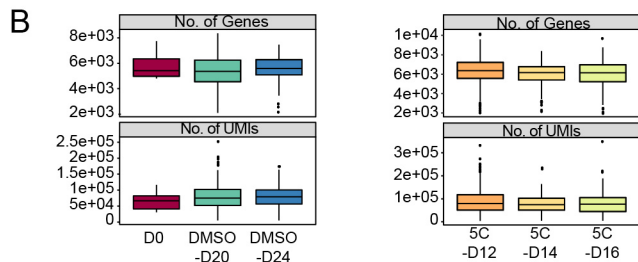
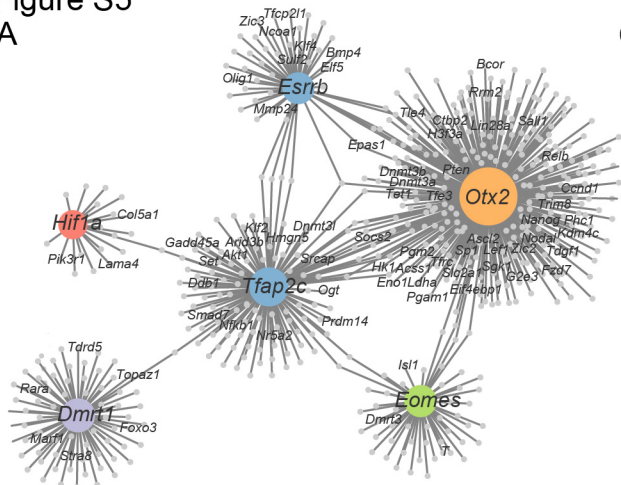
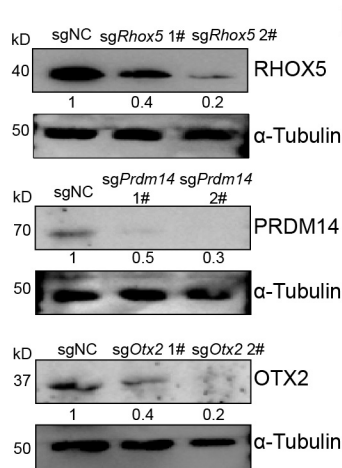


Figure S5

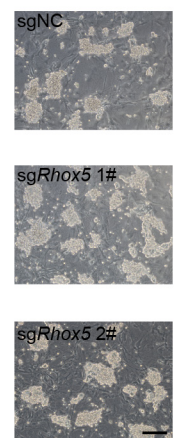
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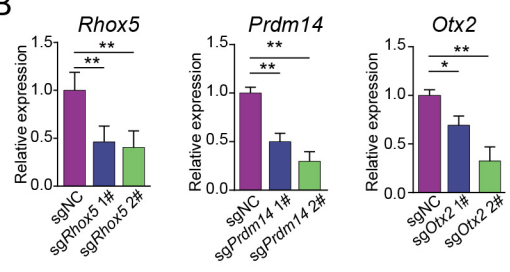
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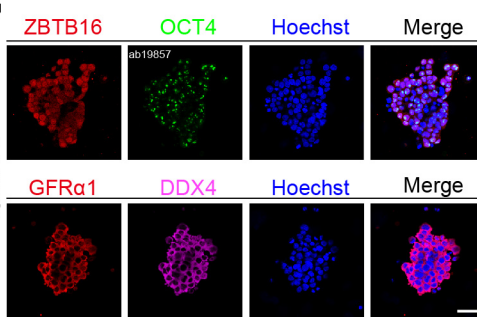
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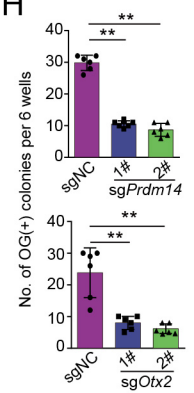
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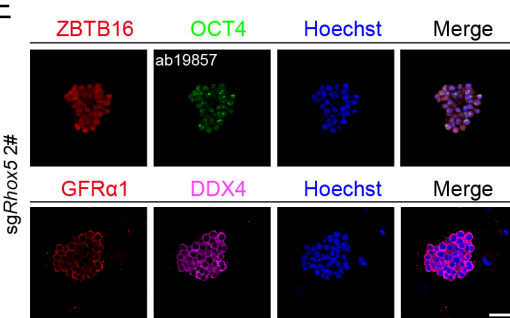
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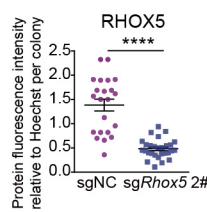
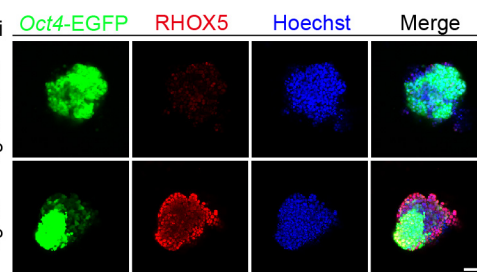
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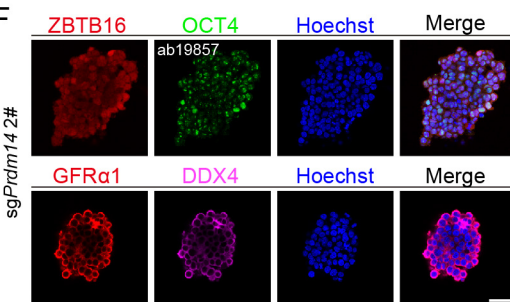
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I



F



ii

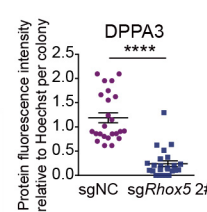
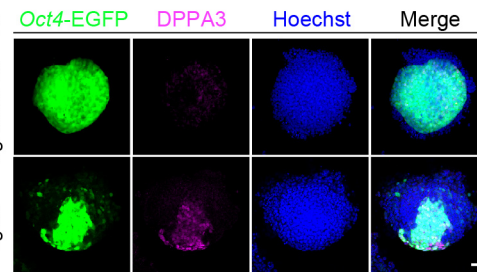
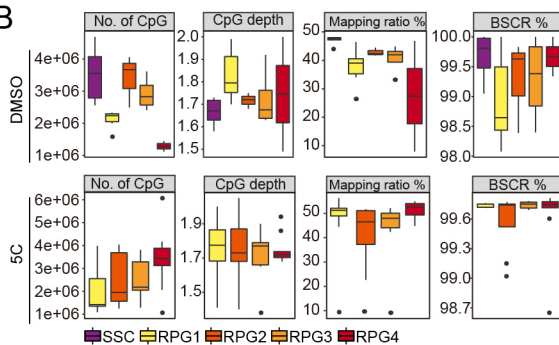


Figure S6

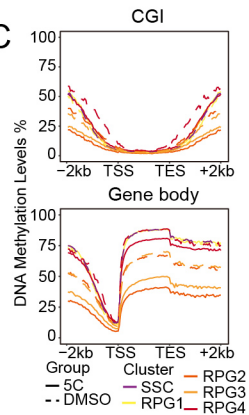
A

Numbers of single cells for scTrio-seq2 (DNA methylation)						
DMSO	SSC	RPG1	RPG2	RPG3	RPG4	
Cell number	6	6	3	4	2	
5C	RPG1	RPG2	RPG3	RPG4		
Cell number	8	9	9	10		
Total After QC : 57						

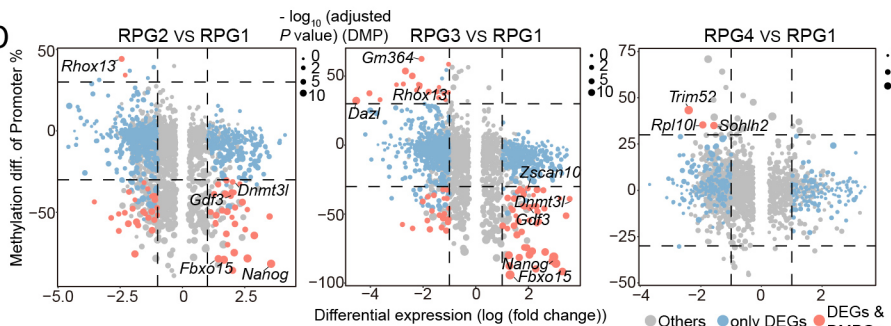
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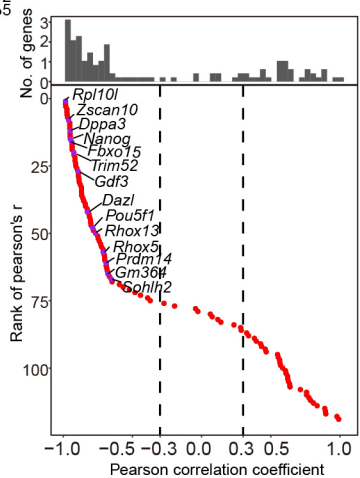
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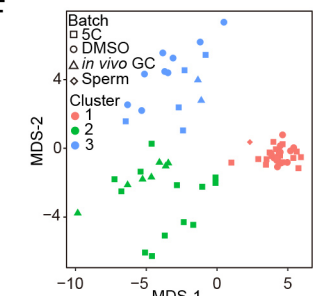
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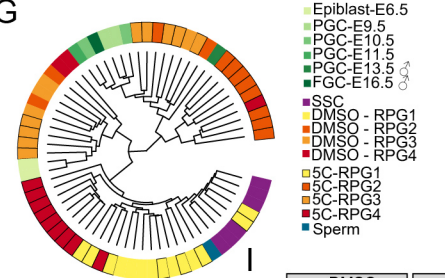
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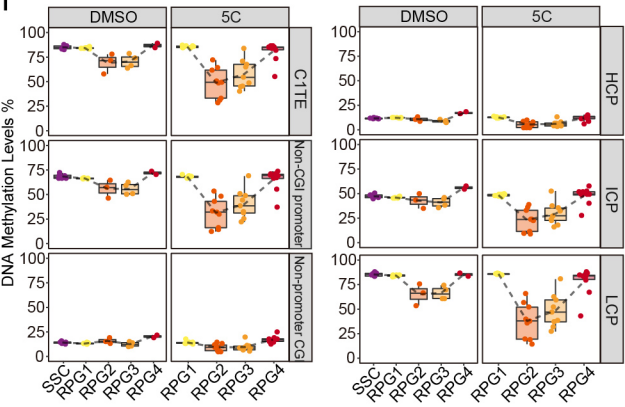
F



G



H



I

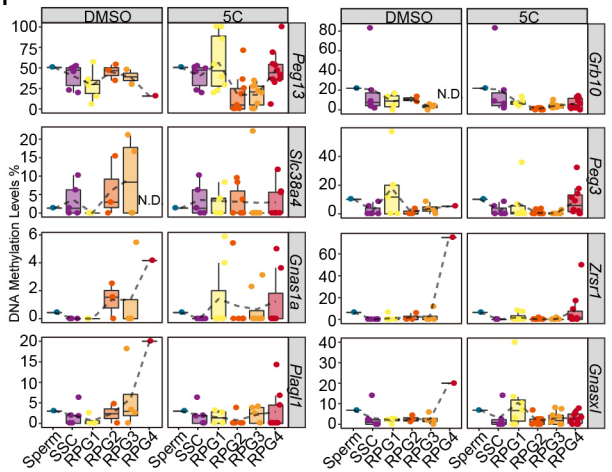
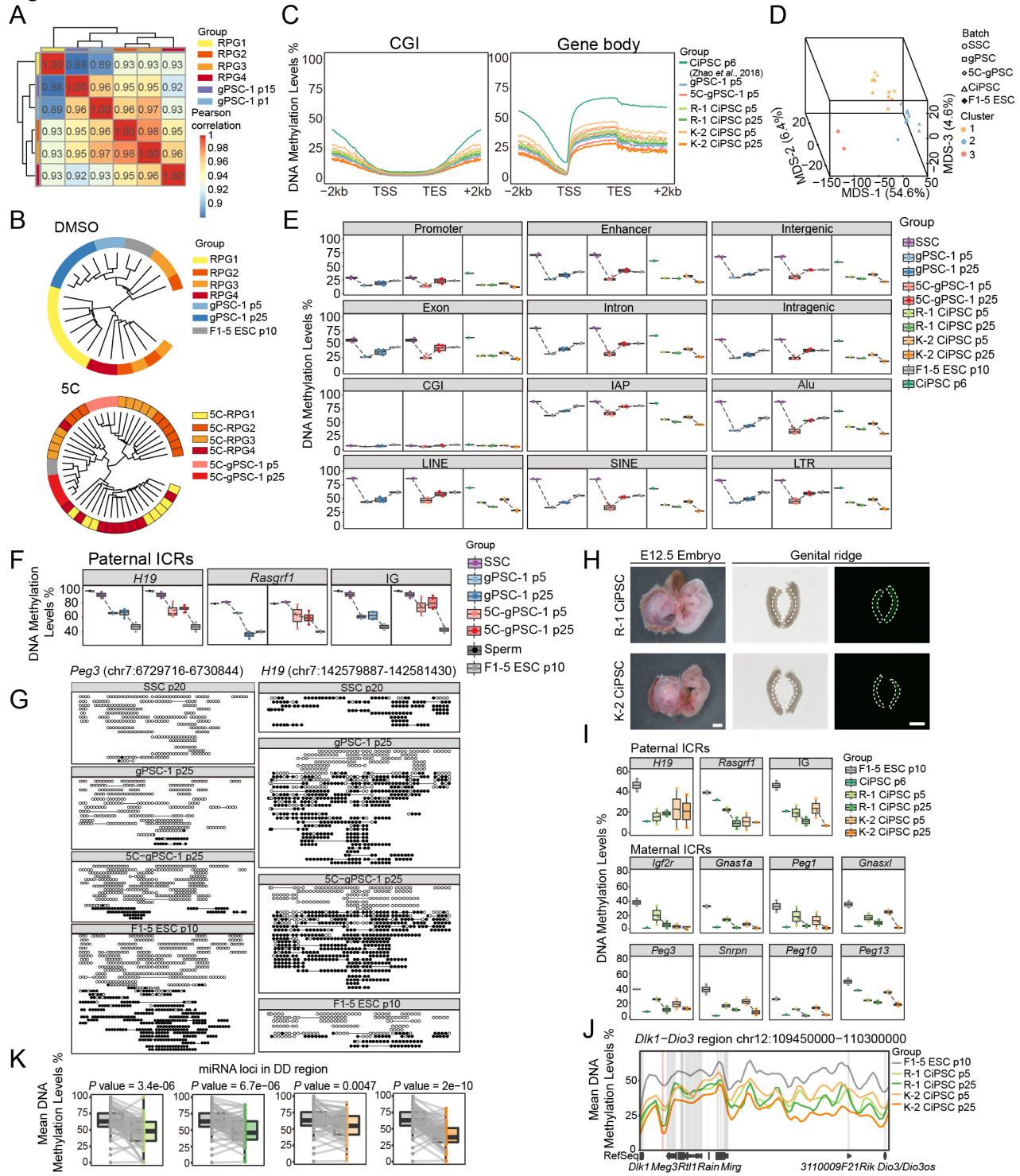


Figure S7



1 **Table S1.** Sample Information of 4,464, 1,089, 57 Cells for scSTRT-seq and scTrio-seq2
2 (transcriptome), scTrio-seq2 (DNA methylation), respectively, related to Figures 1C and 6A,
3 Figures S1J, S1K, S4A, S4C, and S6A.
4

5 **Table S2.** DEGs and GO Terms of Four Clusters in the Successful and Failed Reprogramming
6 Branches, respectively, related to Figure 1H and Figure S2F. Genes sets used to calculate gene
7 signatures in Figure S2H.
8

9 **Table S3.** DEGs and GO Terms of Two Stages amongst SSC Reprogramming and Two MEF-
10 reprogramming Processes, related to Figures 1M and 1N, Figures S2I and S2J.
11

12 **Table S4.** Candidate compounds for chemical screening, Primers for SSLP, Genotyping and
13 q-PCR. Guide RNAs for Gene Knockdown, related to Figures 2A, 3B, 3C, 3O, and 5D, Figure
14 S5B.
15

16 **Table S5.** GO Terms of 836 Stage-specific Genes of *in vivo* Germ Cell Expressed in the
17 Corresponding Clusters of SSC Reprogramming, related to Figure 4C.
18

19 **Table S6.** DEGs and SCENIC Analysis of Each Cluster in the Successful Reprogramming
20 Branch, related to Figures 5A, 5B and 5C, Figure S5A.
21

22 **Table S7.** DEGs and DMPGs of RPG1/2/3/4, related to Figure S6D and S6E.
23

24 **Table S8.** GO Terms of Up-regulated Genes of RPG2 and RPG3 from 5C Group than that from
25 Control and DMSO Groups, related to Figure 6H.