Dissecting gene regulation network in human early embryos at single-cell and single-base resolution

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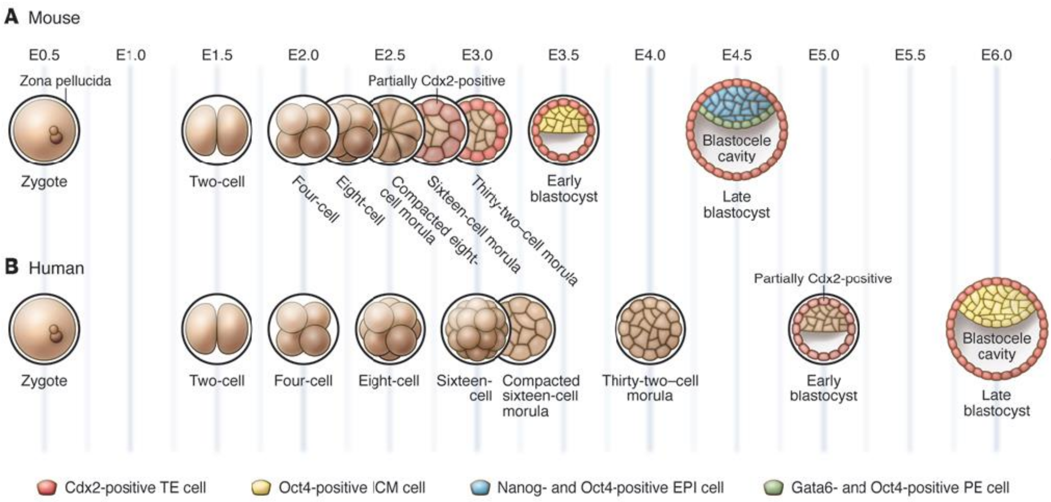


Cockburn and Rossant, 2010

Mammalian embryonic development starts from a single cell - zygote

Single Cell Transcriptome

Single Cell Genome

Low number of Cells Epigenome

In vivo

In vitro

In vivo

In vivo

In vitro

Key SNVs

Key Transcription Factors

Crucial epigenetic codes

Gene Expression Network

Epigenetic Network

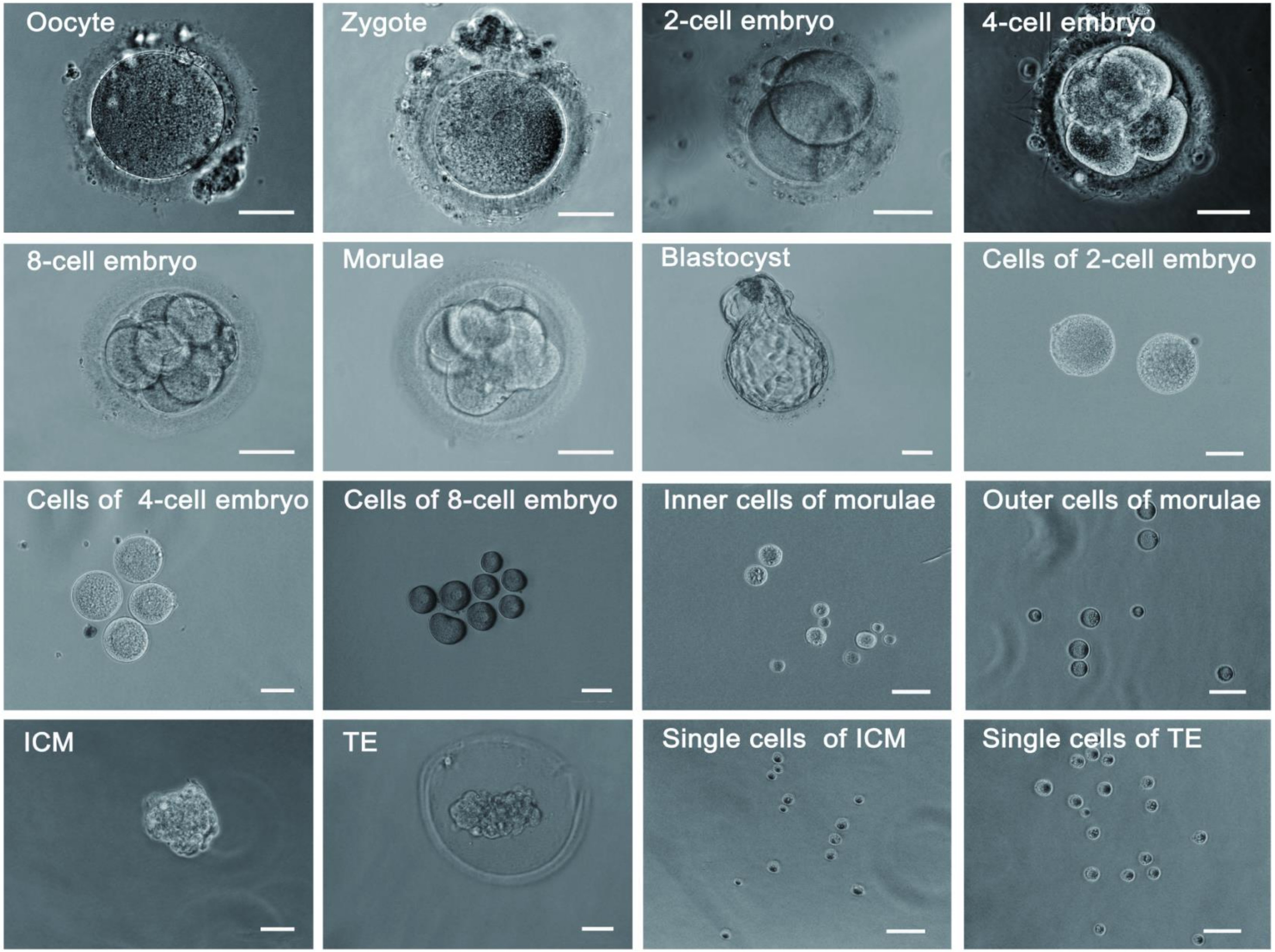
Cell Fate Determination
(Early Embryonic Development)

Mouse Model
(Gene Knockout & Knockdown)

Human ES Cell Model
(Gene Knockdown & Overexpression)

Early Embryo

Dissecting Gene Regulation Network of Human Early Embryos



Isolation of single cells from human preimplantation embryos

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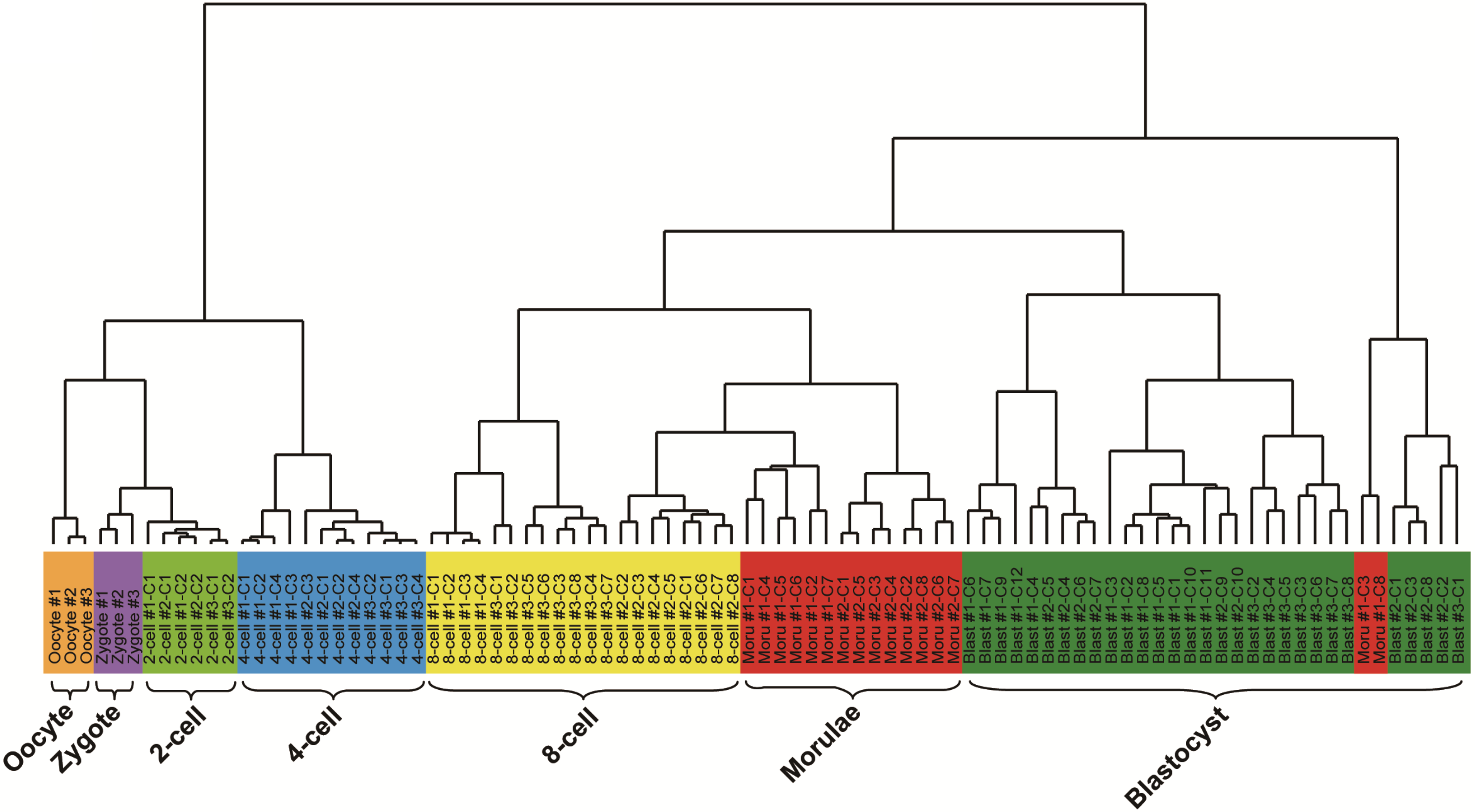
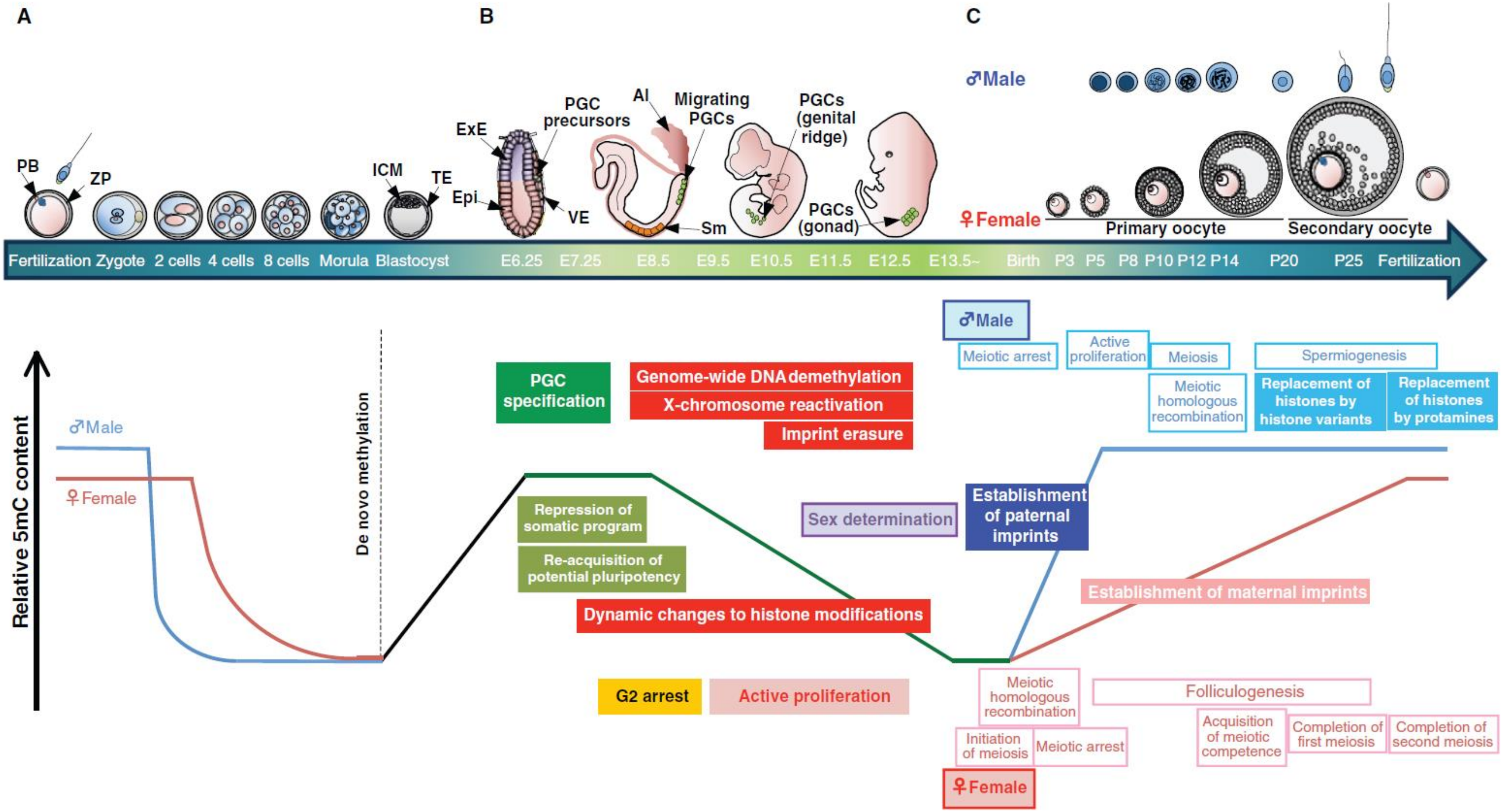


- **An individual human embryonic cell expresses:**

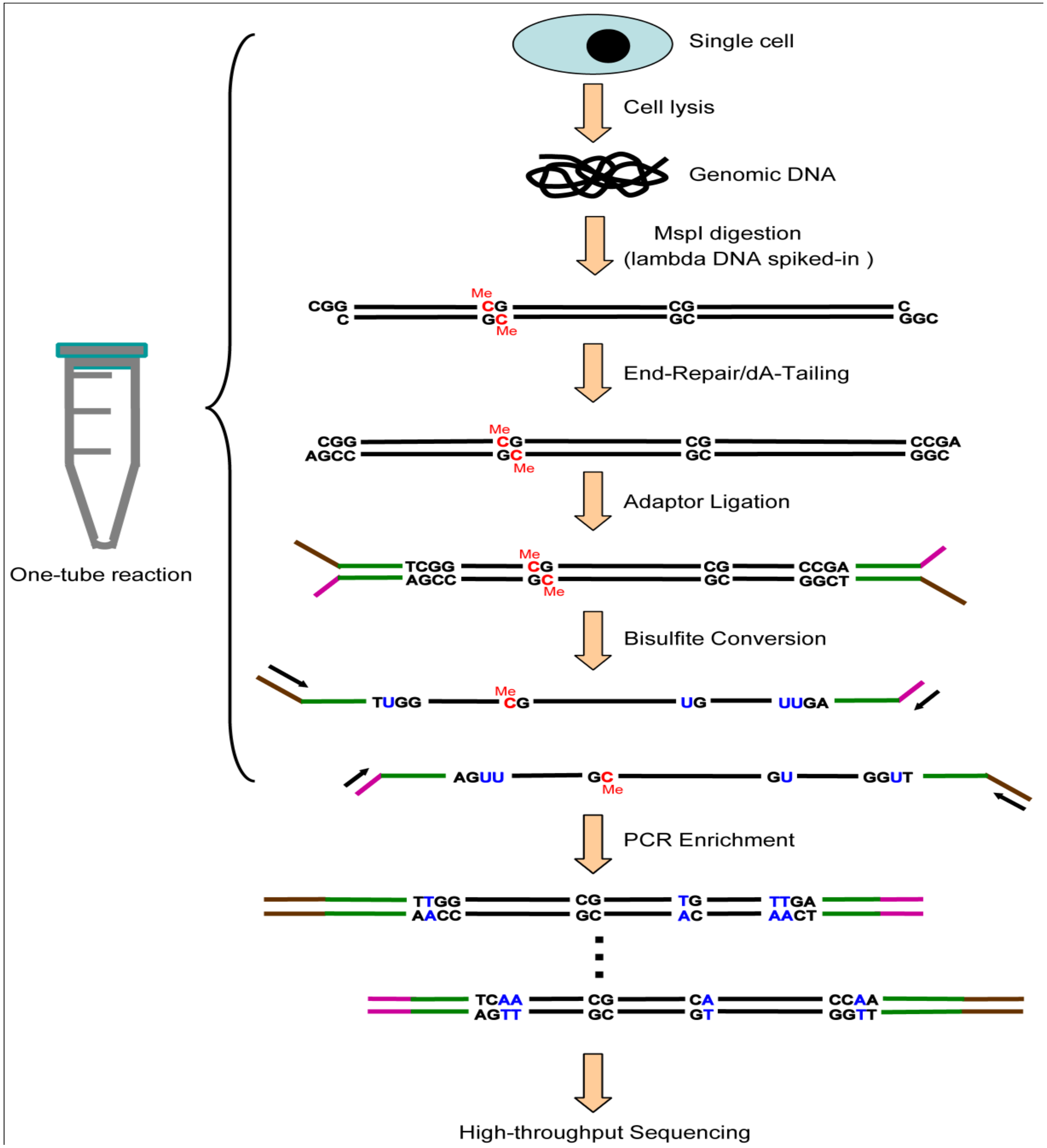
  18,022 RefSeq transcripts;

  13,772 Ensembl transcripts.

Single cell RNA-Seq of human early embryos
(Yan et al., Nature Structural & Molecular Biology, 2013)



Global DNA methylome changes during mammalian embryonic development (Saitou et al., Development 2012)



Single cell

Cell lysis

Genomic DNA

MspI digestion
(lambda DNA spiked-in)

End-Repair/dA-Tailing

Adaptor Ligation

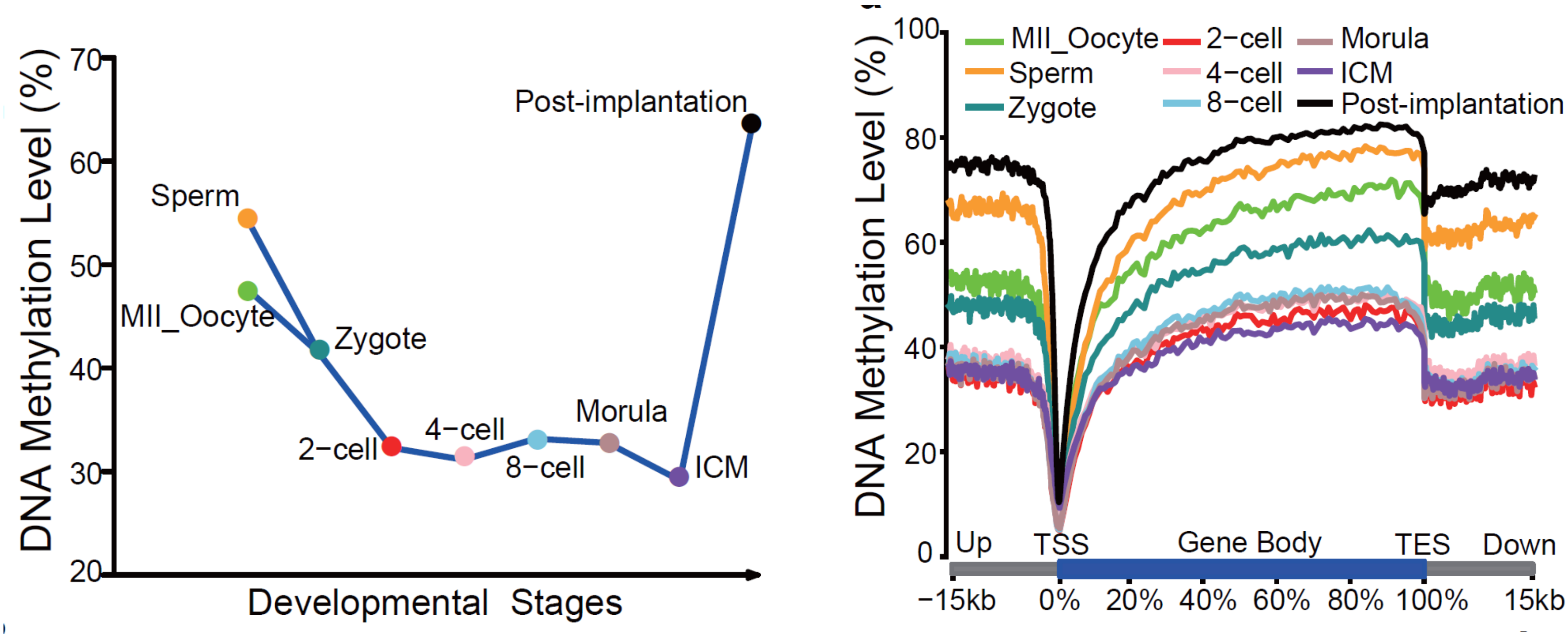
Bisulfite Conversion

PCR Enrichment

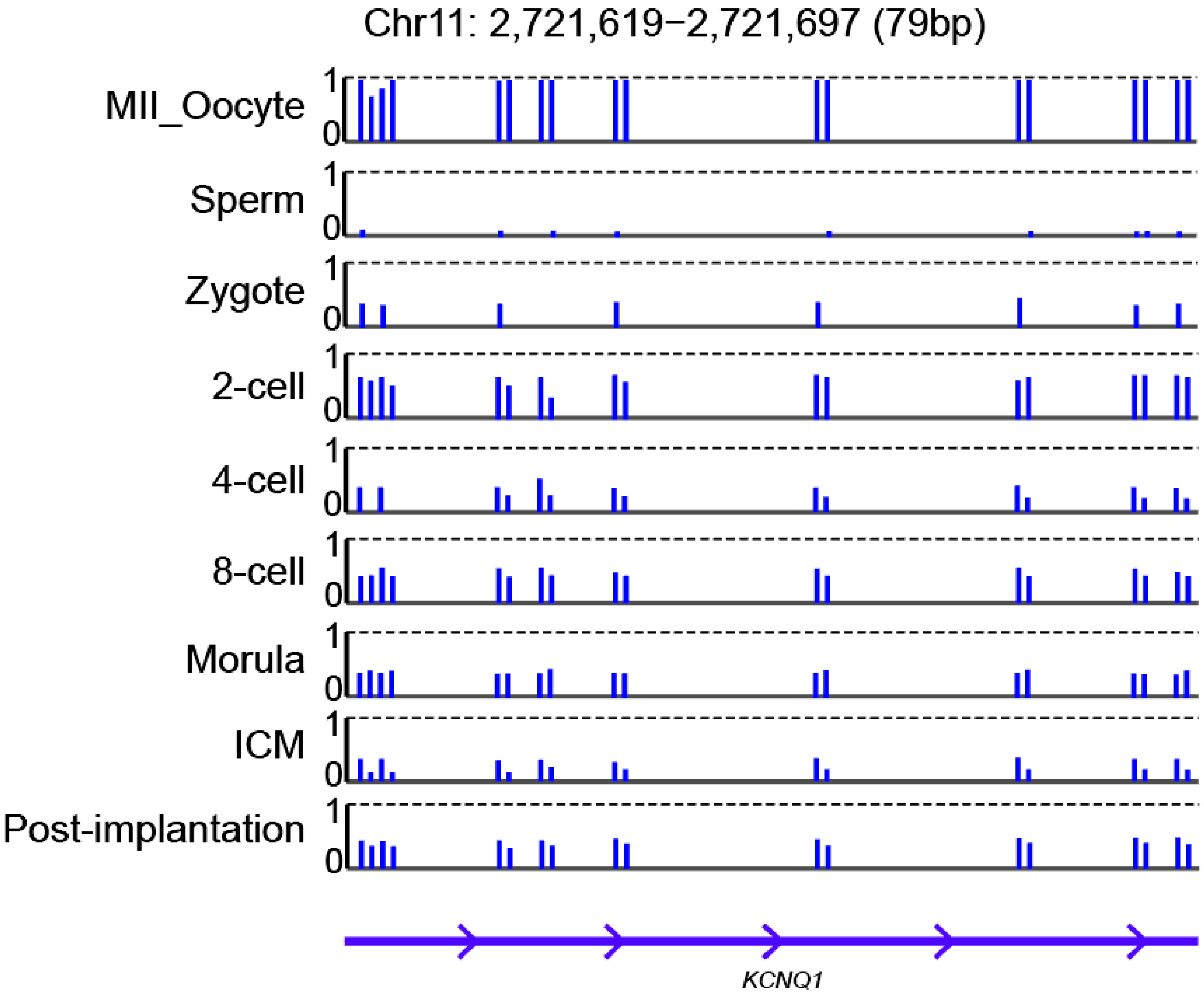
High-throughput Sequencing

One-tube reaction

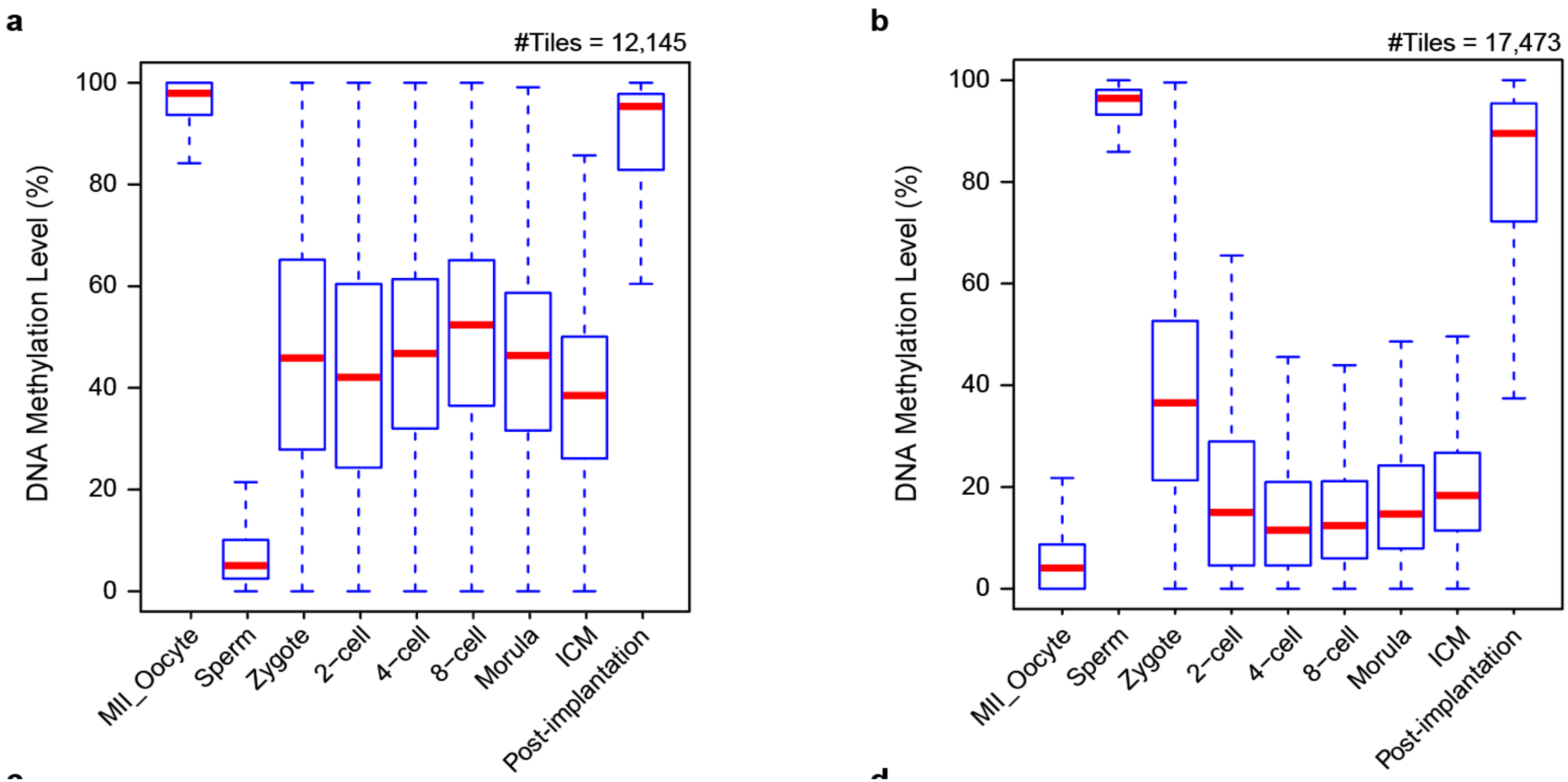
Single cell DNA methylome analysis (Guo et al., Genome Research, 2013)



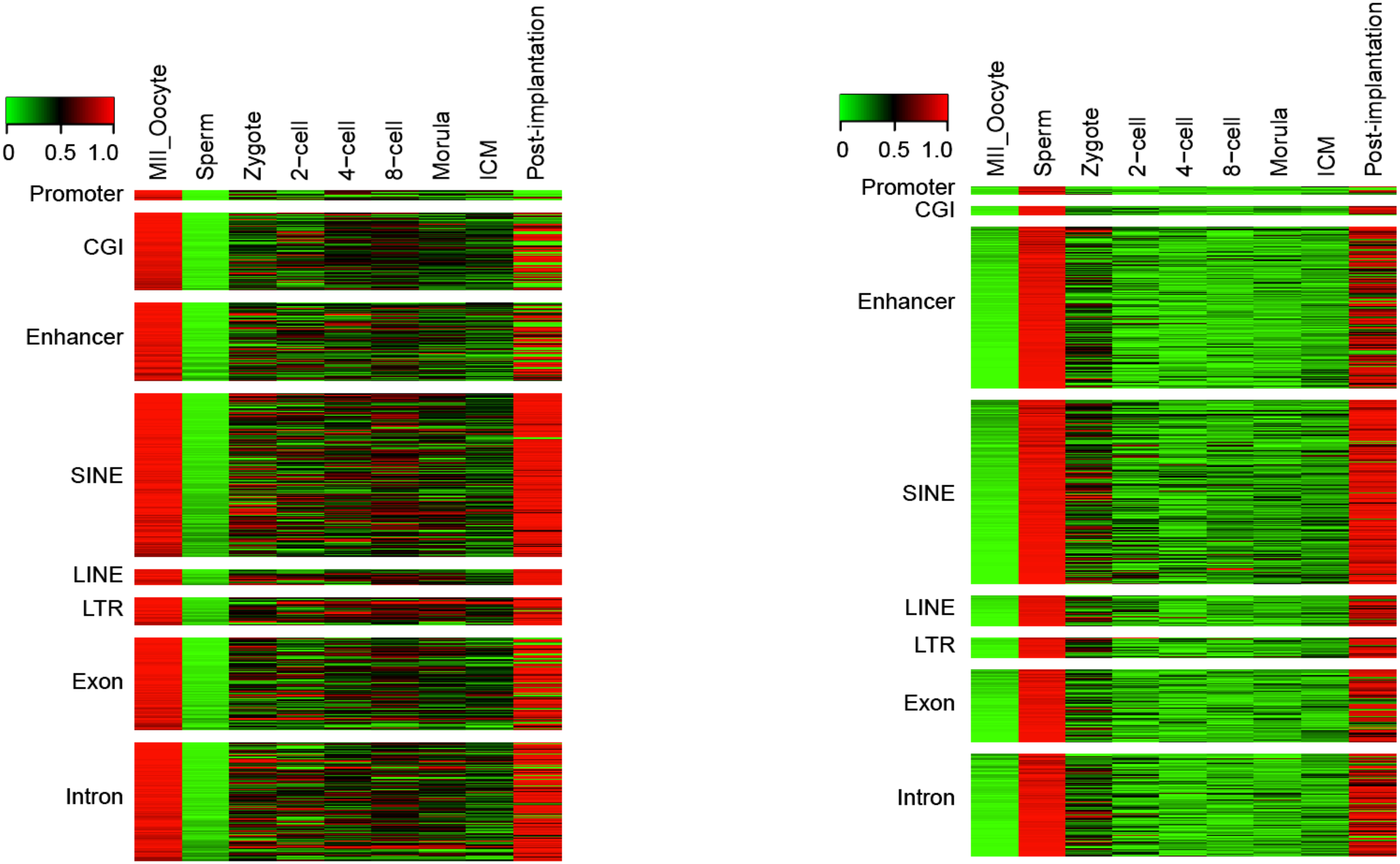
Global DNA methylation dynamics of human early embryos
(Guo et al., Nature, 2014)



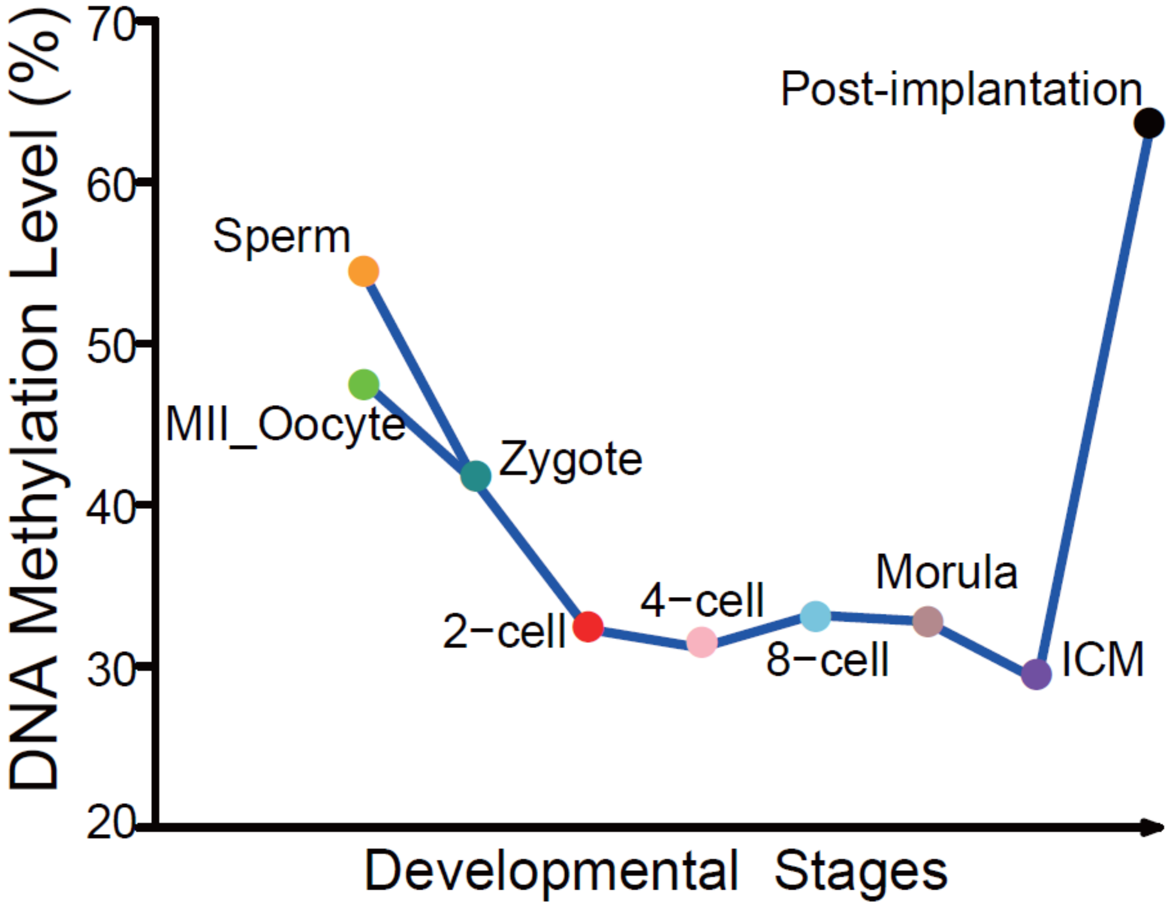
Gene imprinting dynamics of human early embryos



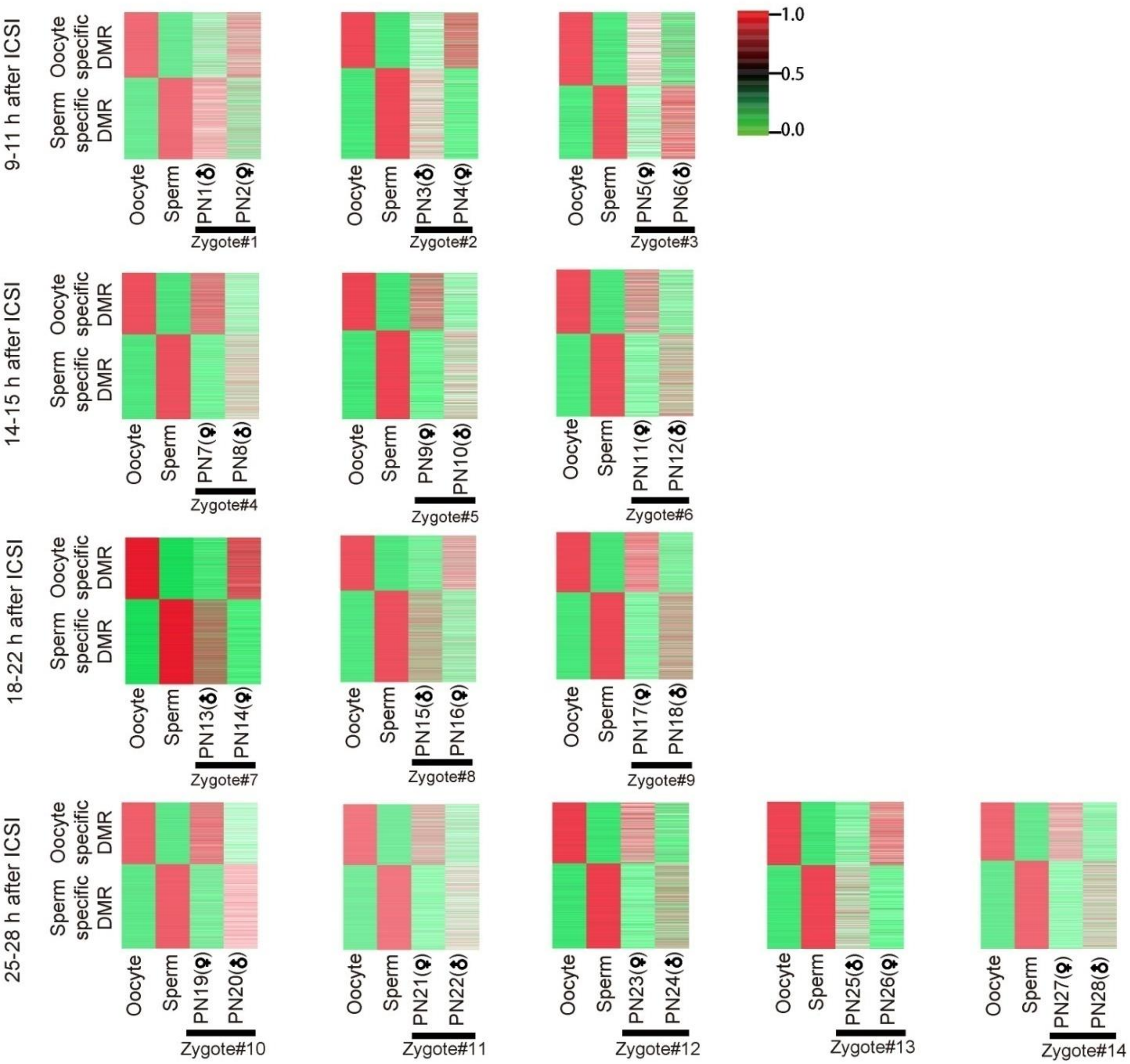
Differentially Methylated Regions (DMRs) of human early embryos



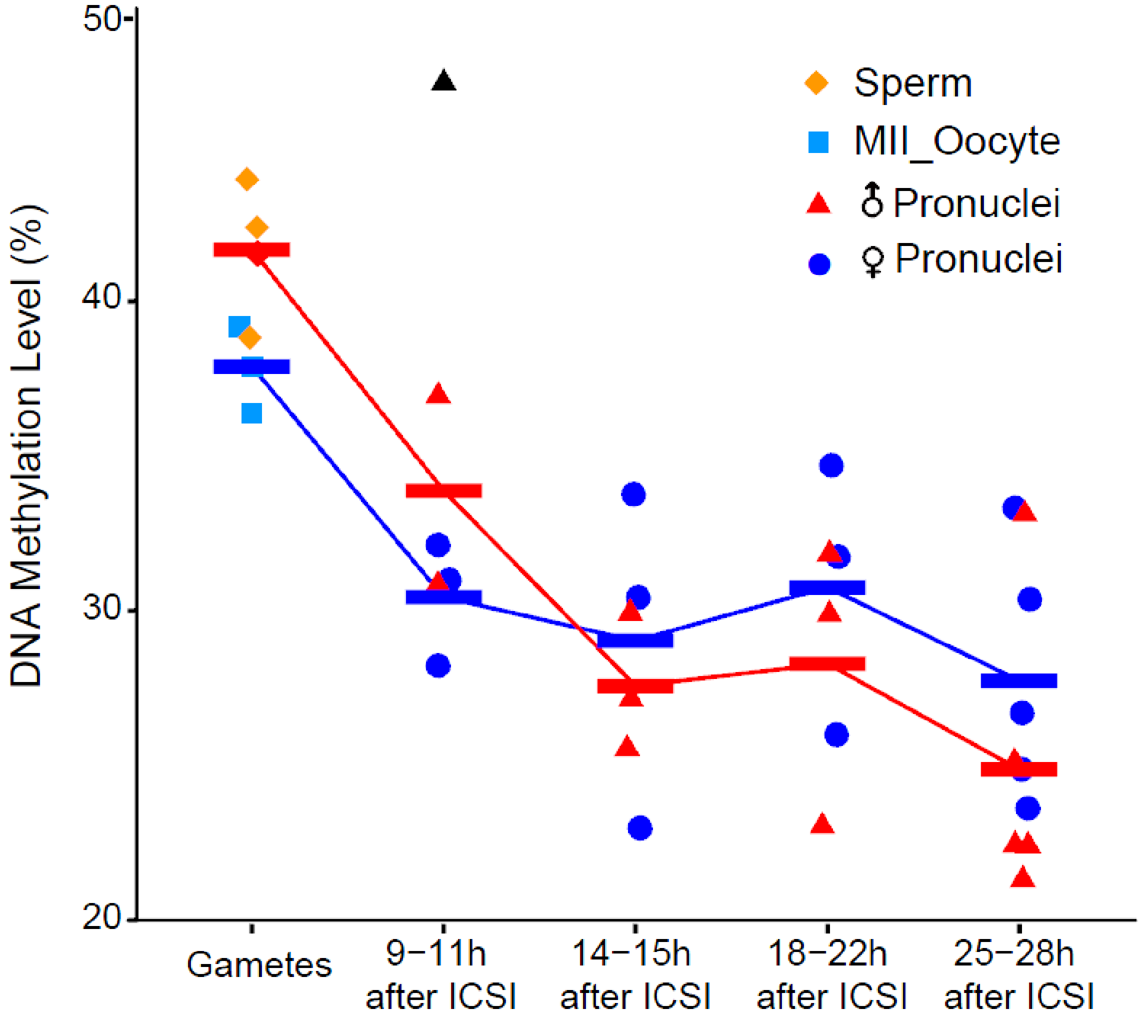
Differentially Methylated Regions (DMRs) of human early embryos



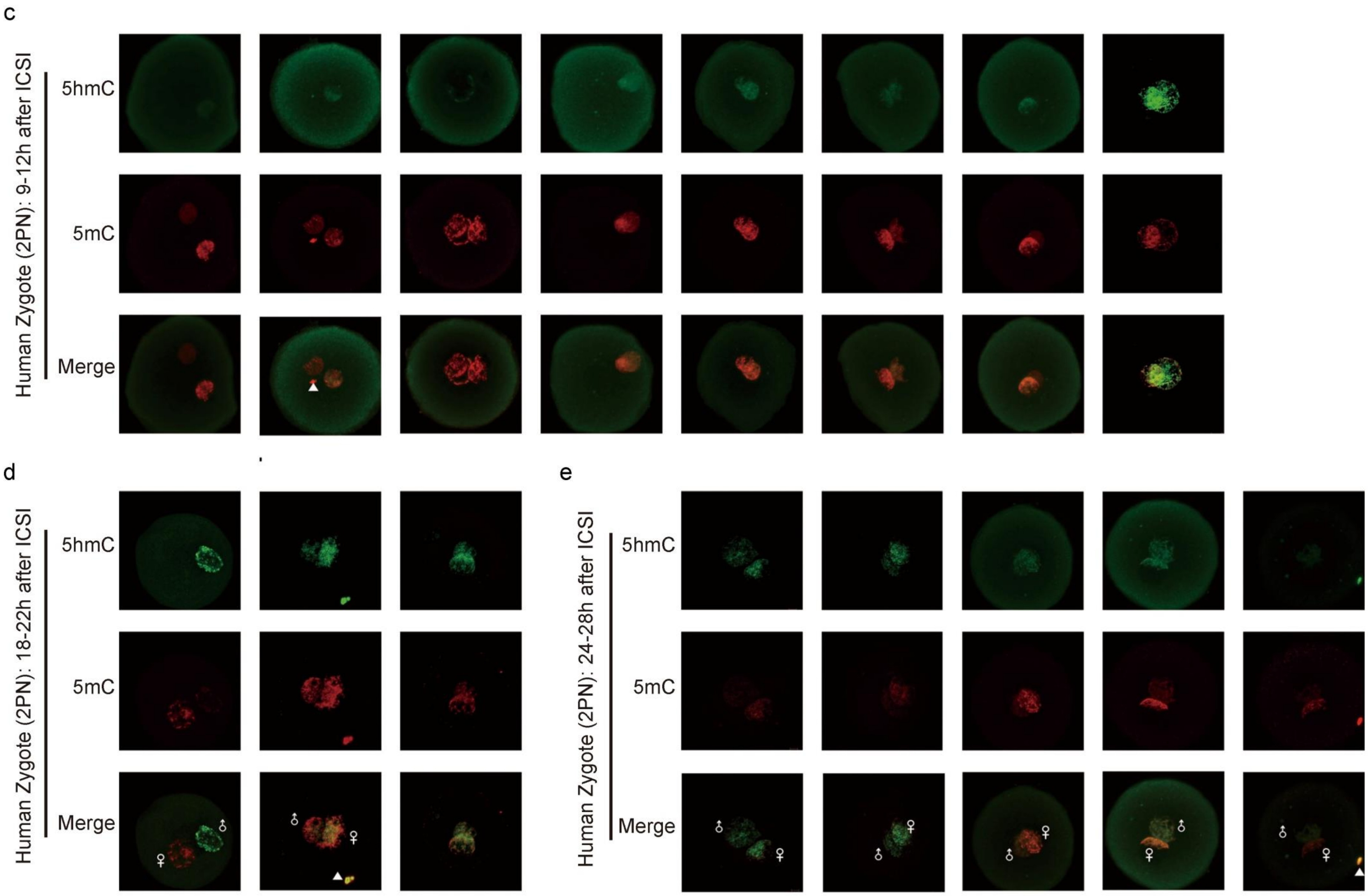
Global DNA methylation dynamics of human early embryos



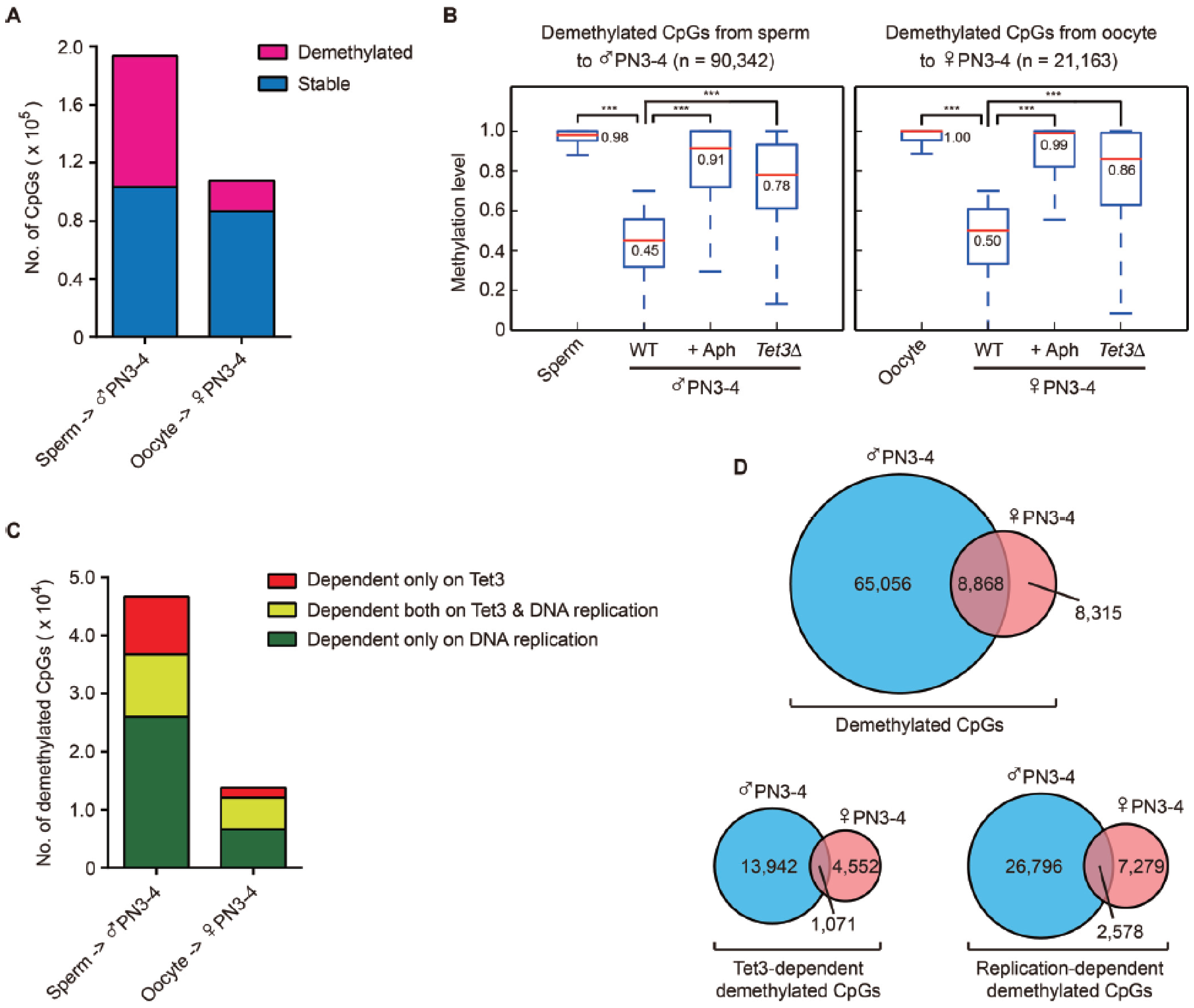
Discriminate male & female pronuclei within individual zygotes



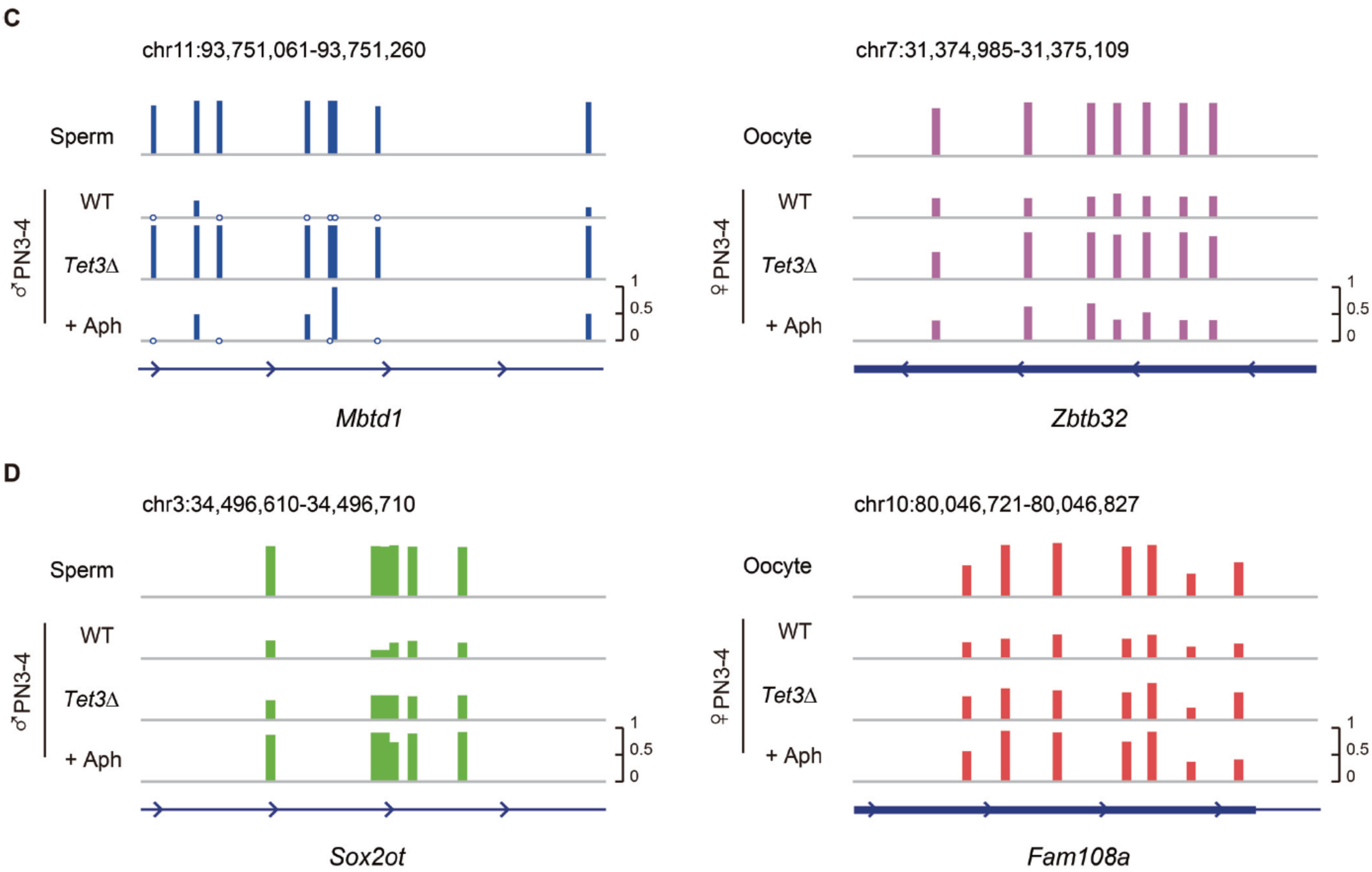
Global DNA methylation at zygote stage within individual pronuclei



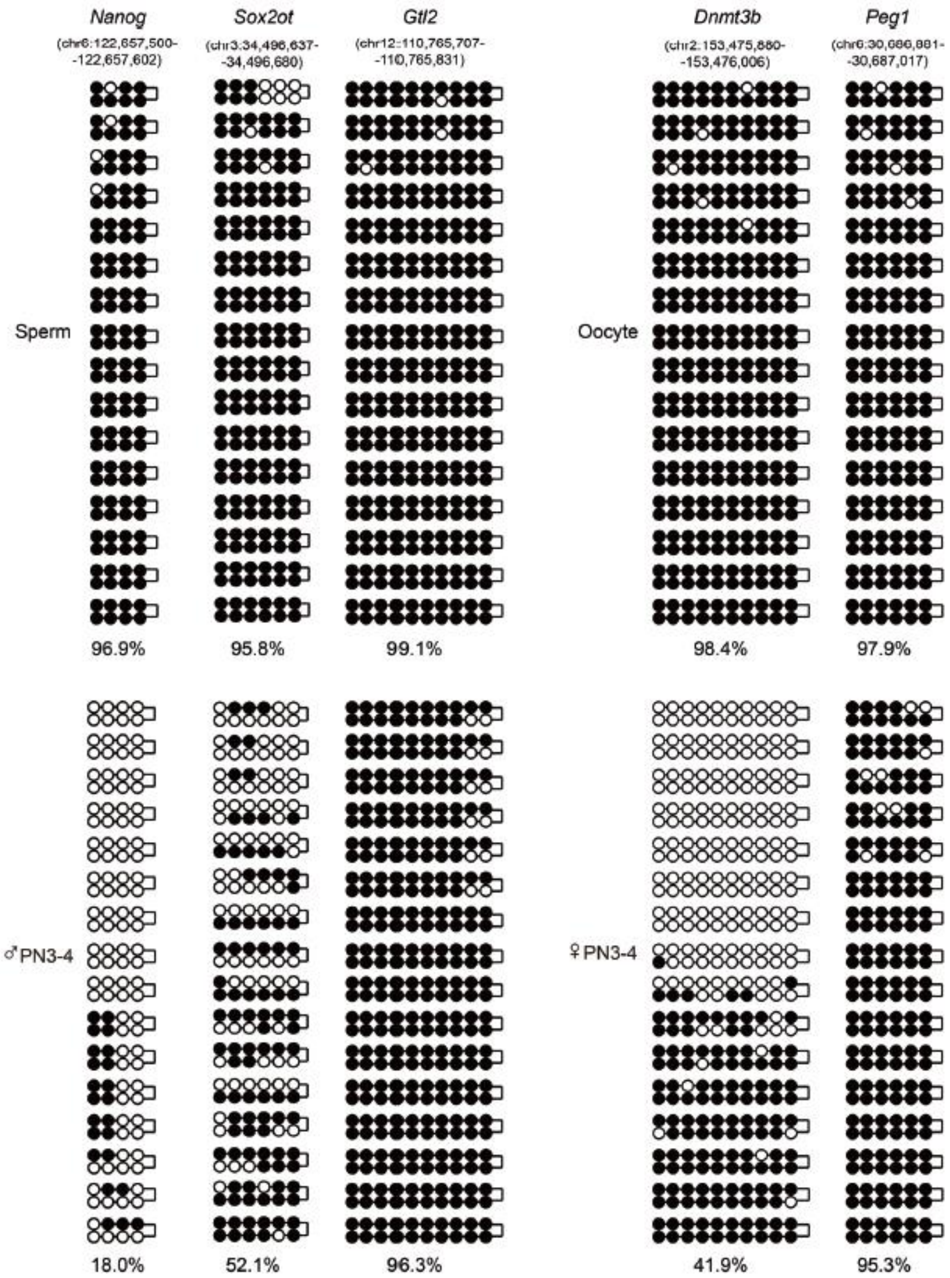
Global DNA methylation at zygote stage within individual pronuclei



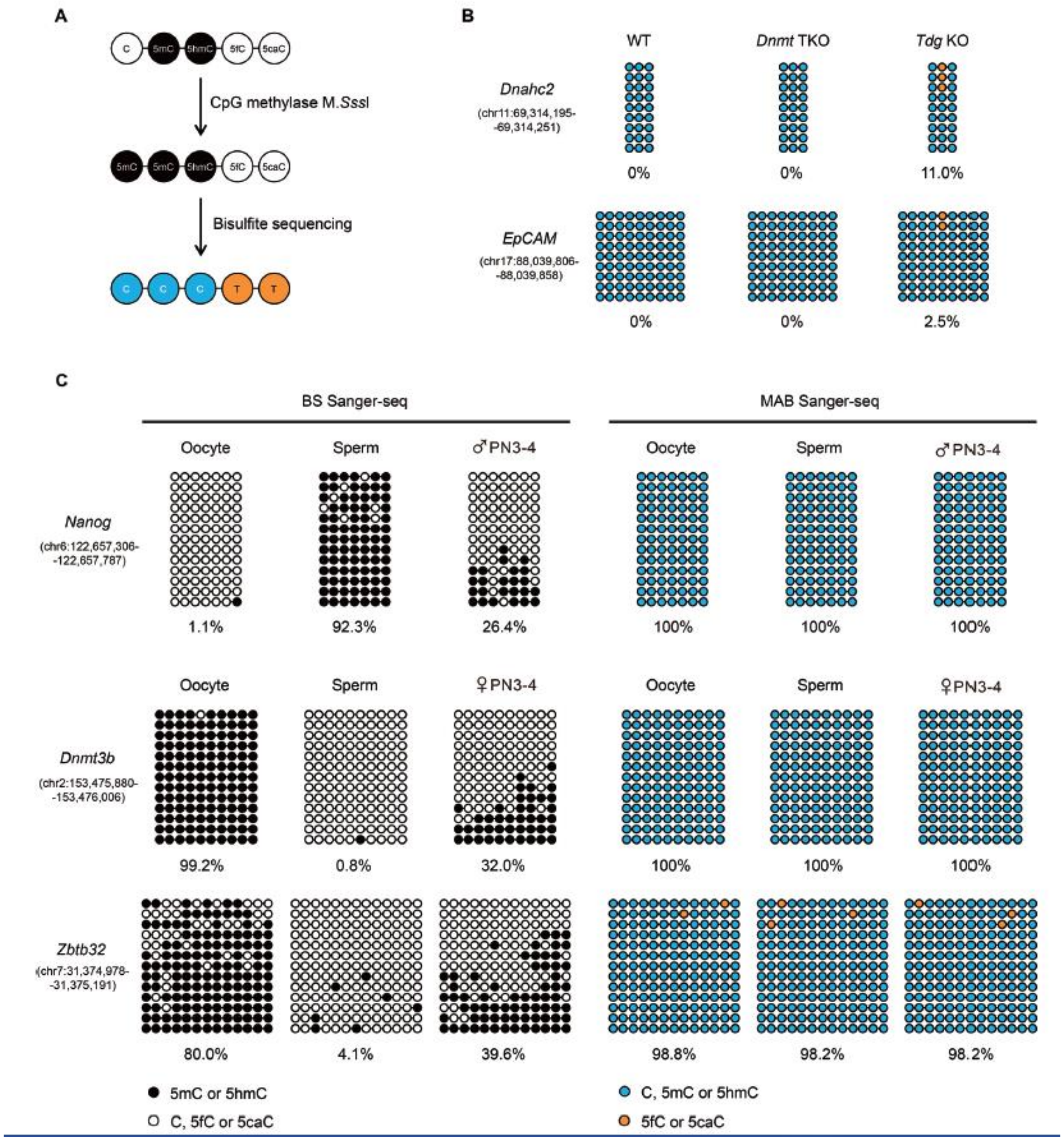
Active DNA demethylation in both maternal & paternal genome in mouse zygotes (Guo et al., Cell Stem Cell, 2014)



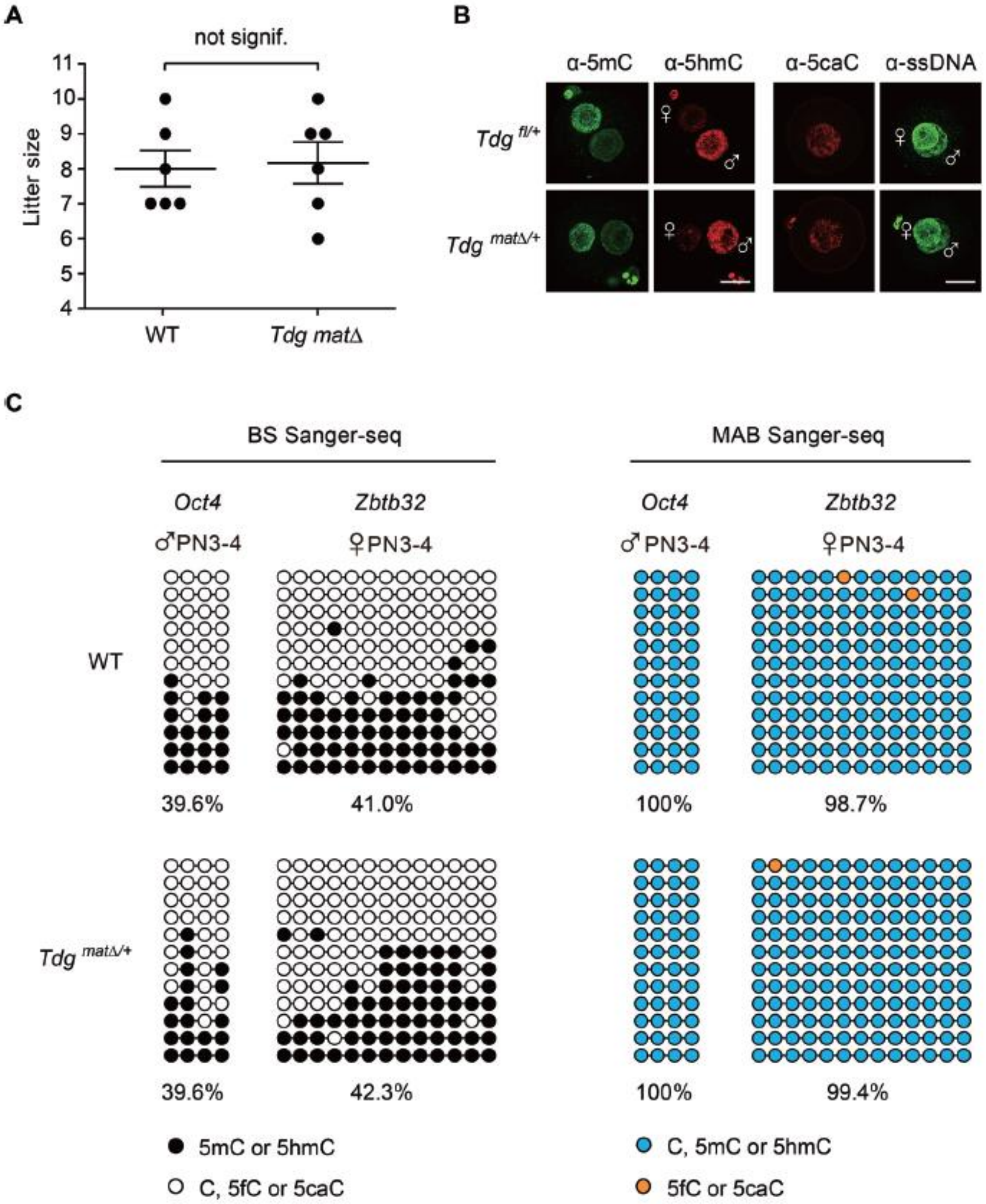
Active DNA demethylation is dependent on Tet3
(Guo et al., Cell Stem Cell, 2014)



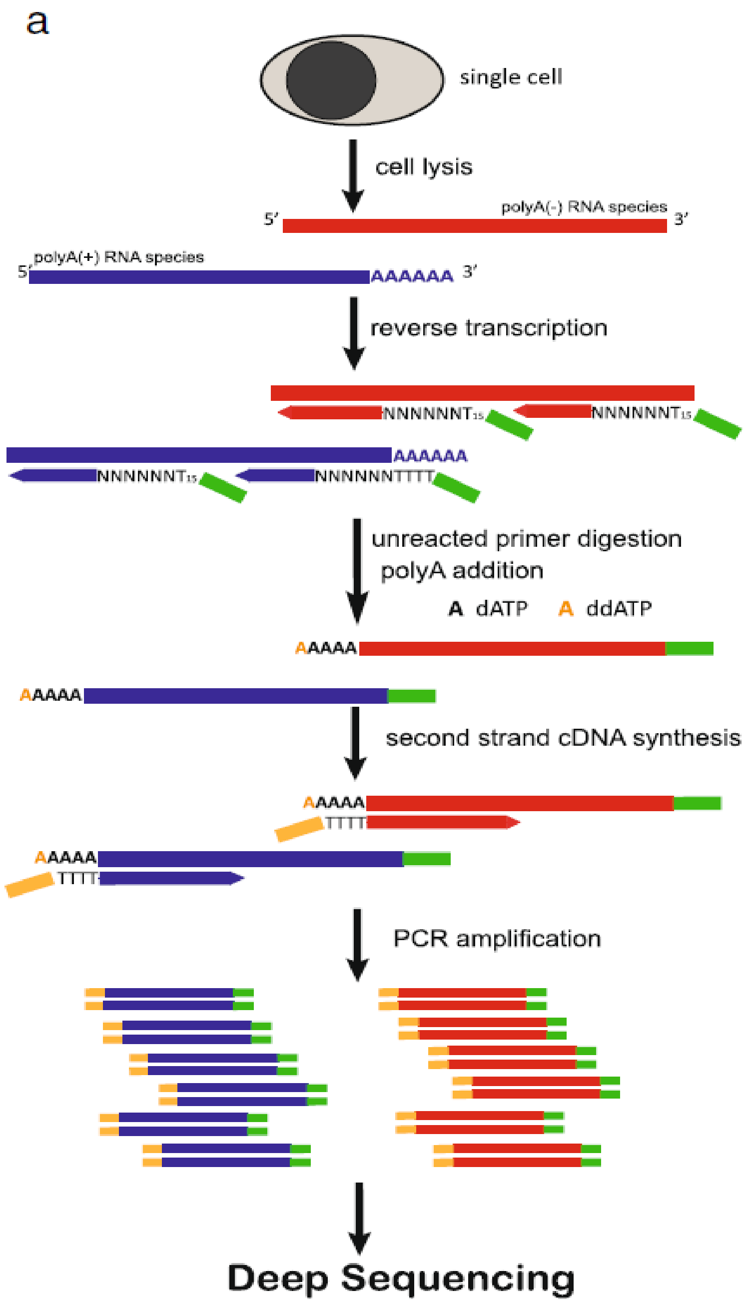
Active DNA demethylation removed 5mCpG on both strands



Active DNA demethylation leads to unmodified cytosines



Active DNA demethylation is independent on TDG



Single cell SUPER-Seq analysis of mouse early embryos
(Fan et al., Genome Biology, 2015)

SUPER-Seq analysis of ‘averaged’ single cells (HEK-293 cells)

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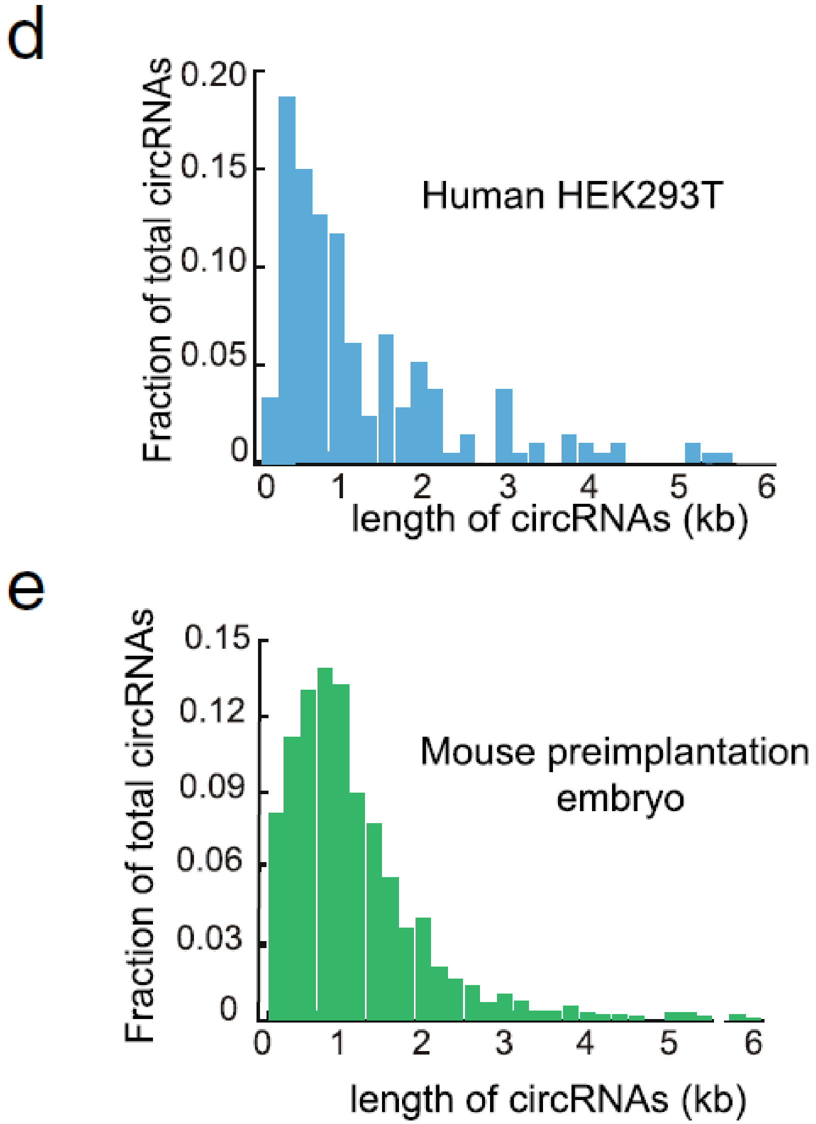
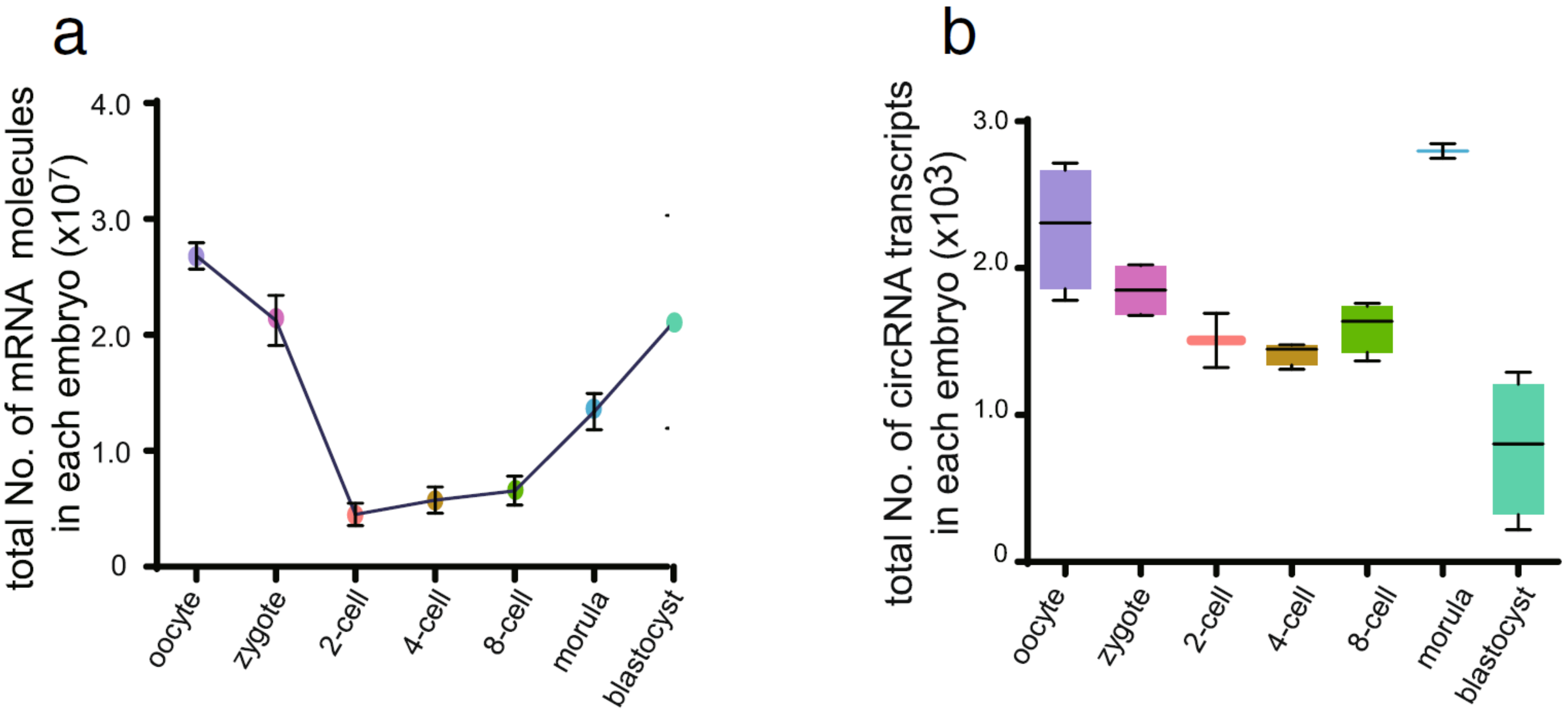


SUPER-Seq analysis to detect circRNAs in mouse preimplantation embryos



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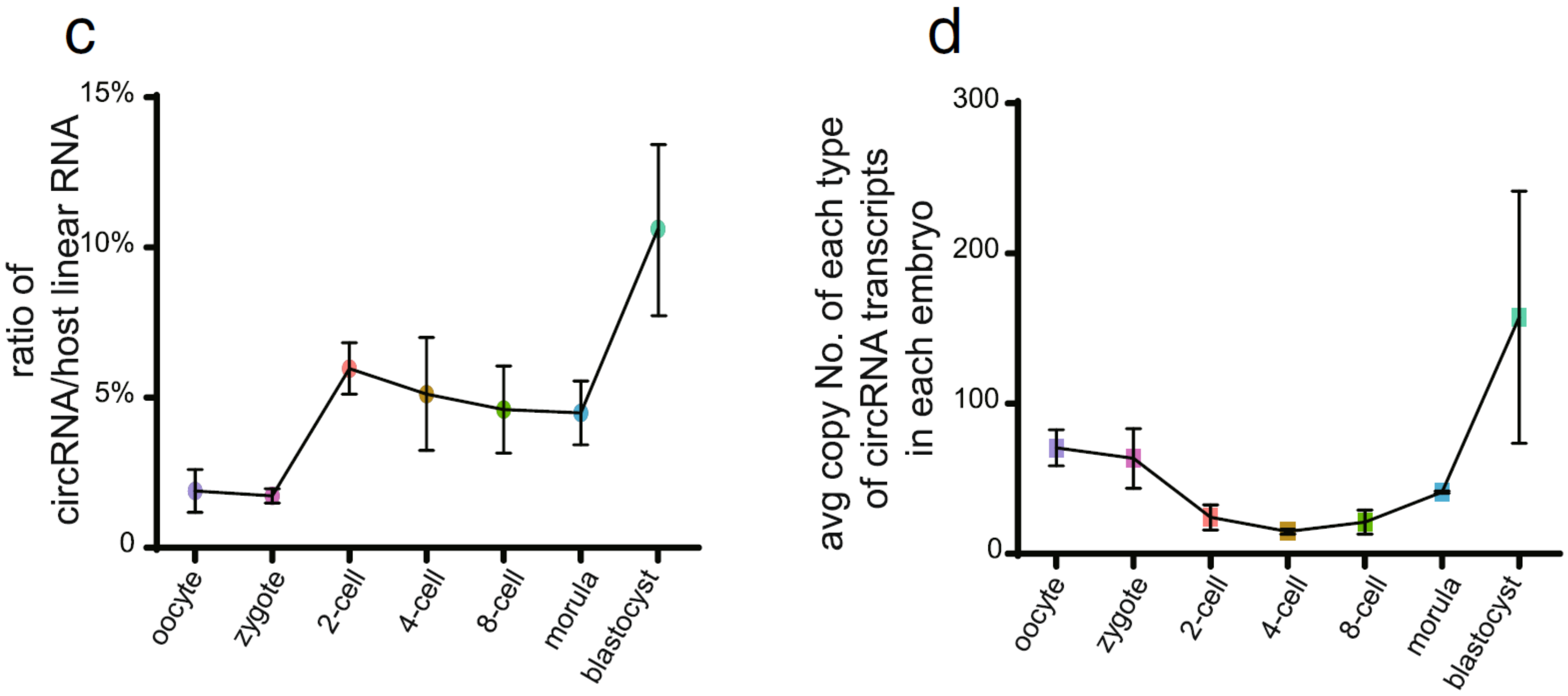

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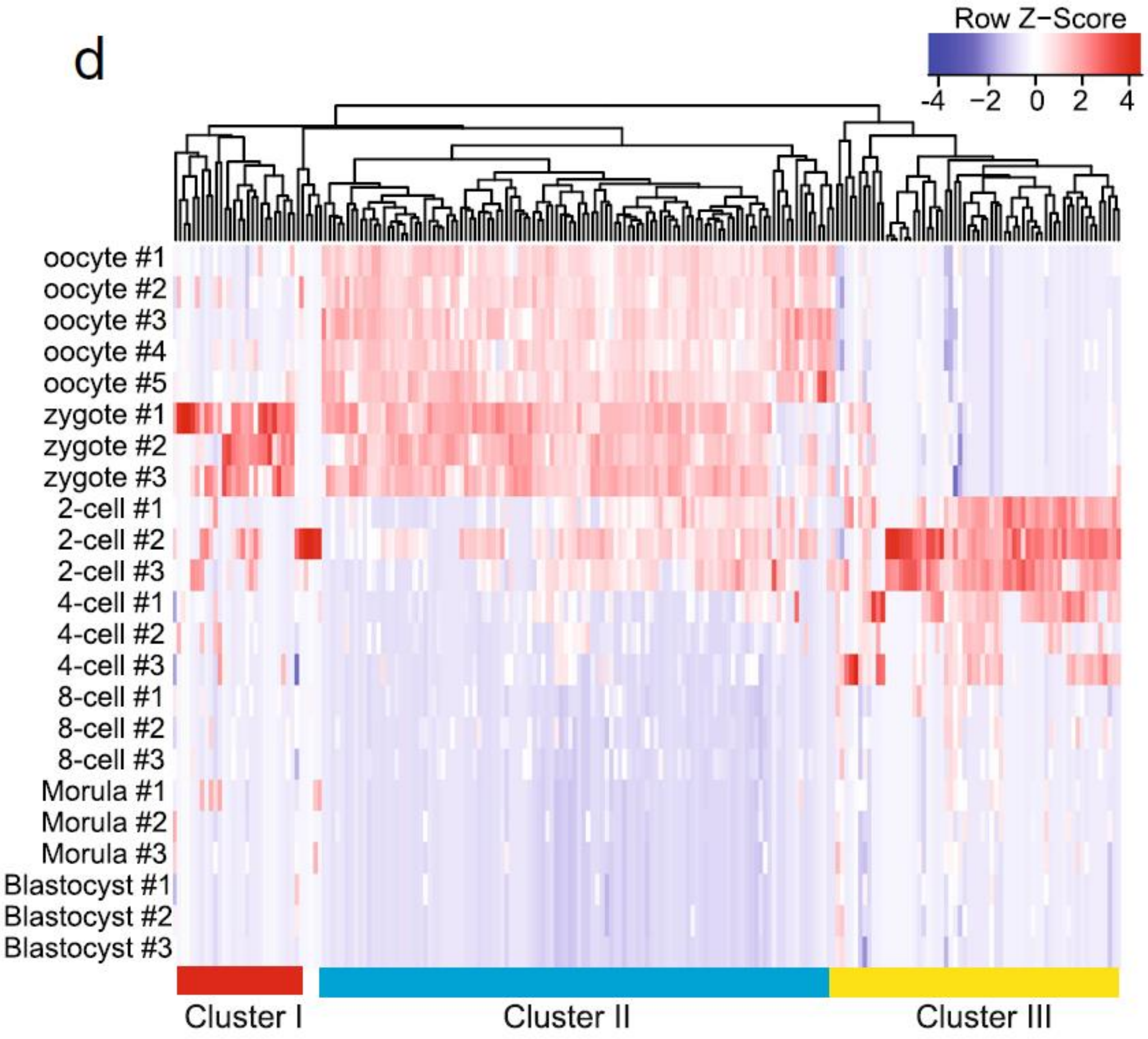
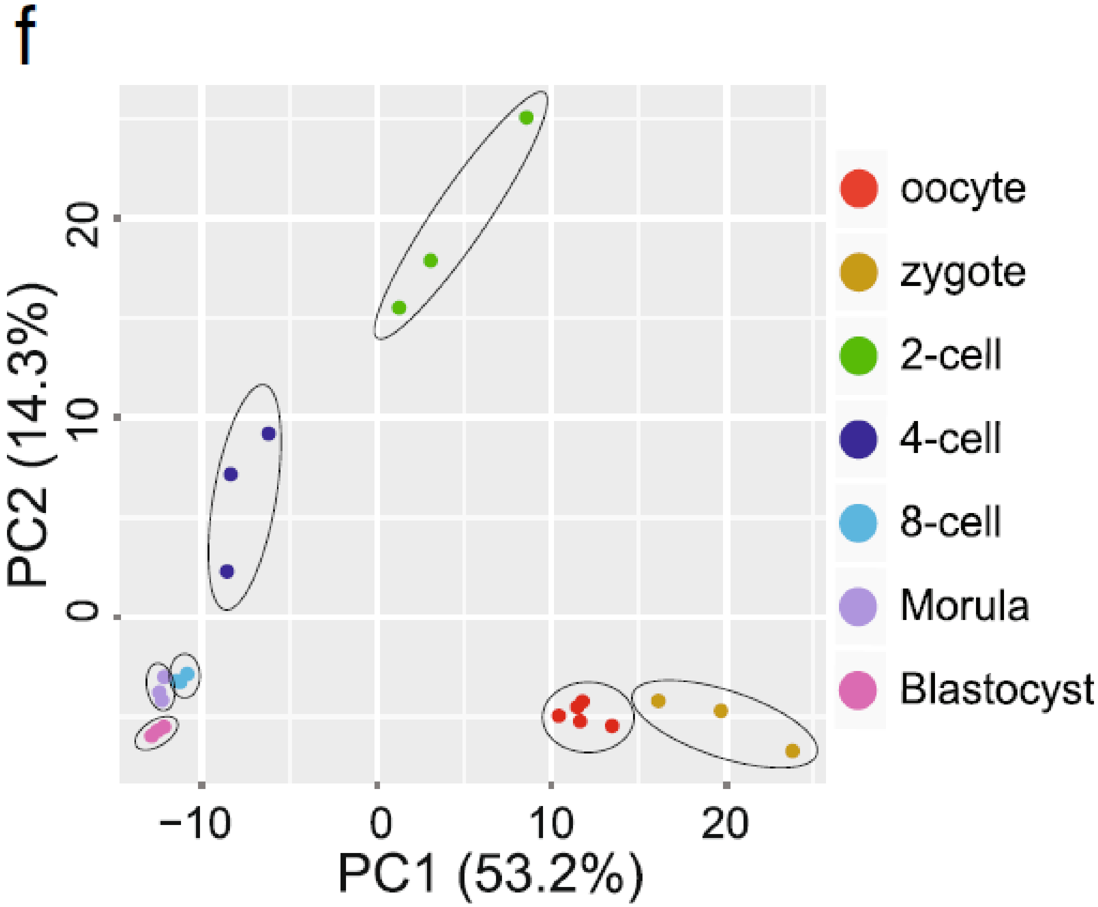
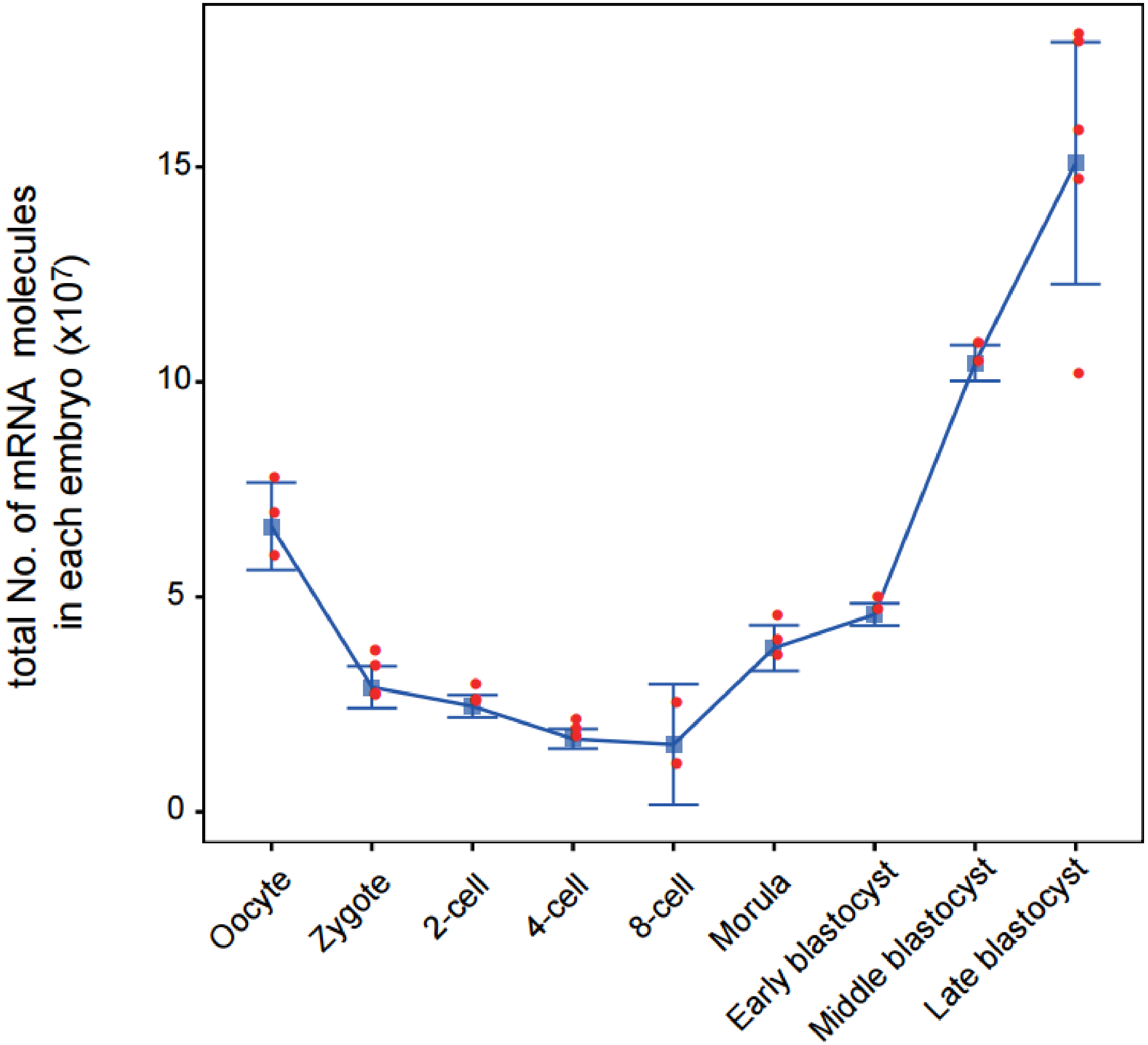


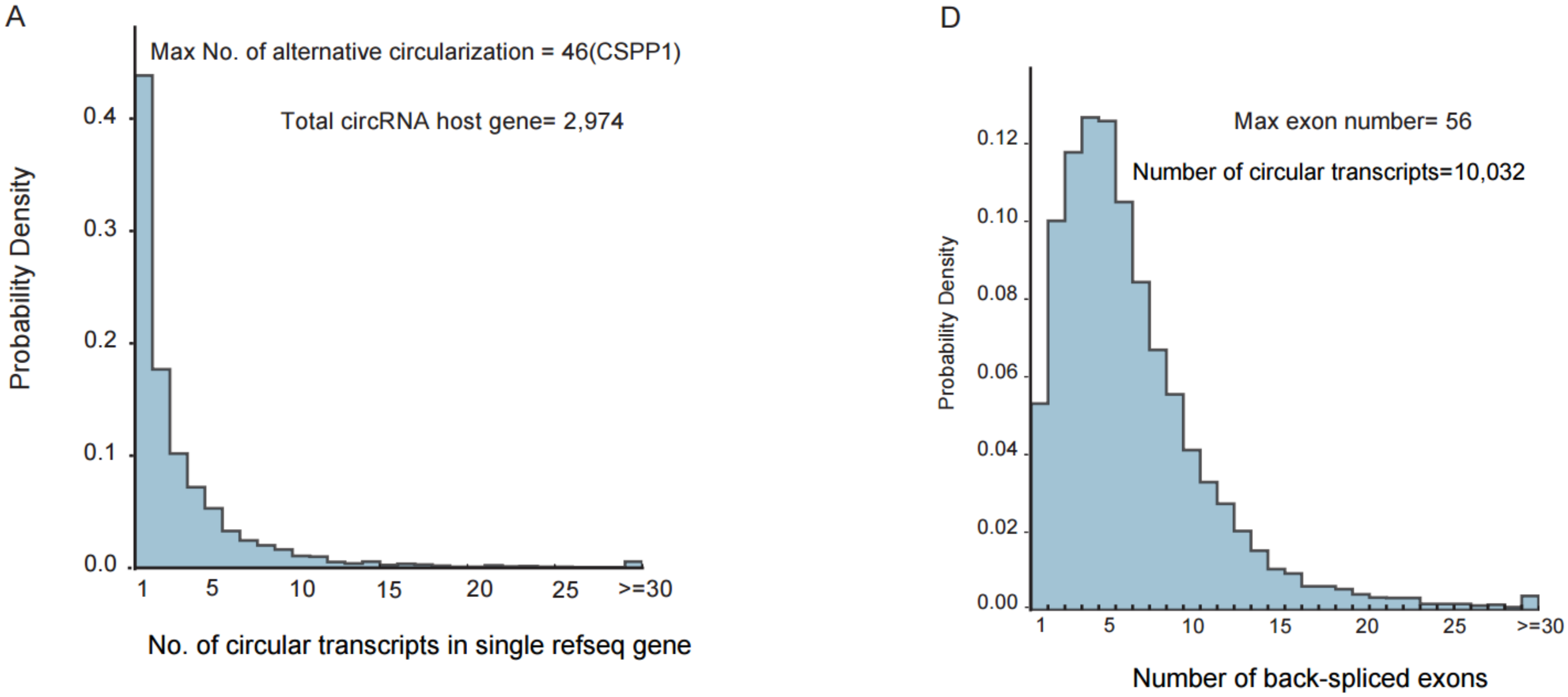
SUPER-Seq analysis to detect novel polyA minus RNAs in mouse preimplantation embryos



SUPER-Seq analysis to detect novel polyA minus RNAs in mouse preimplantation embryos



SUPER-Seq analysis to detect circRNAs in human preimplantation embryos (Unpublished)



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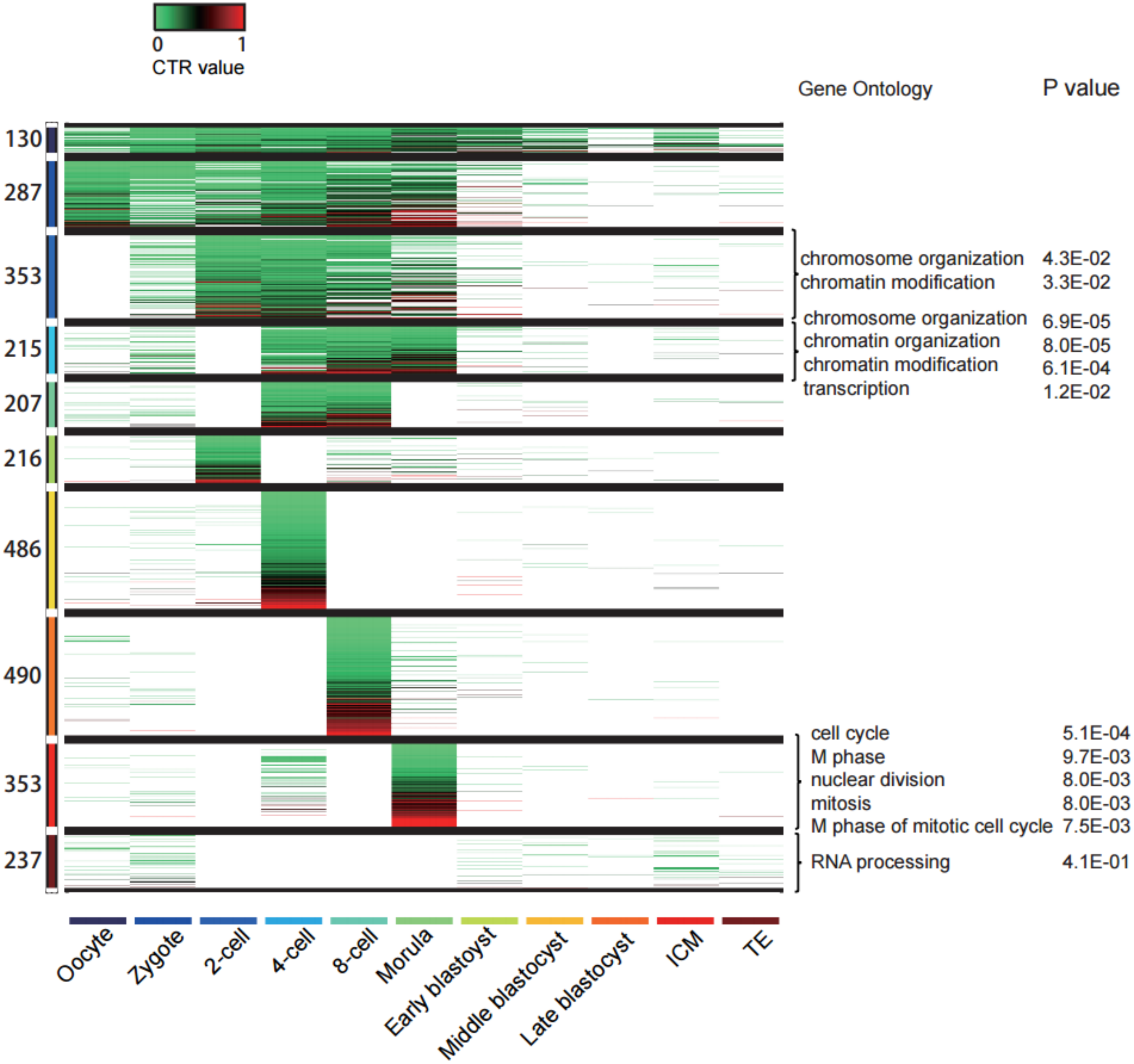

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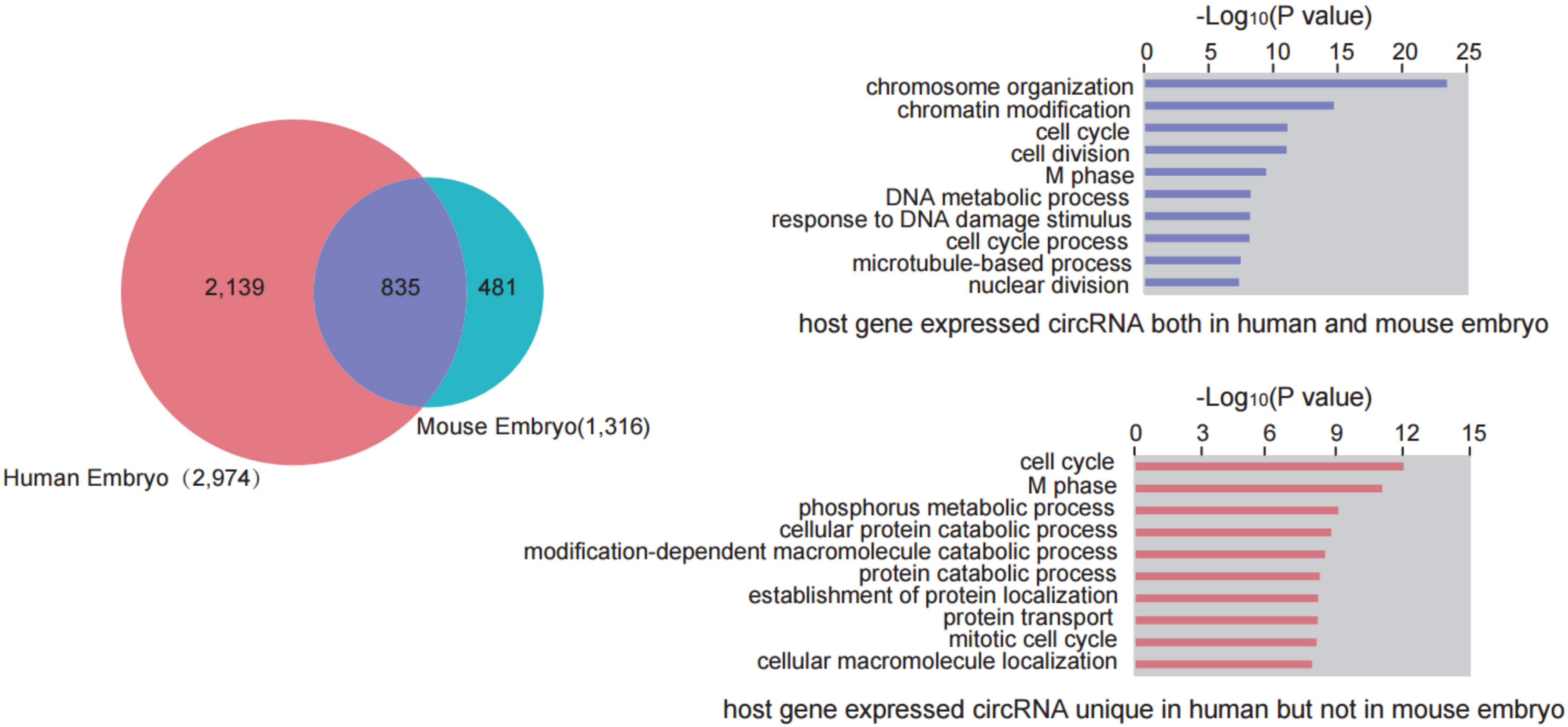

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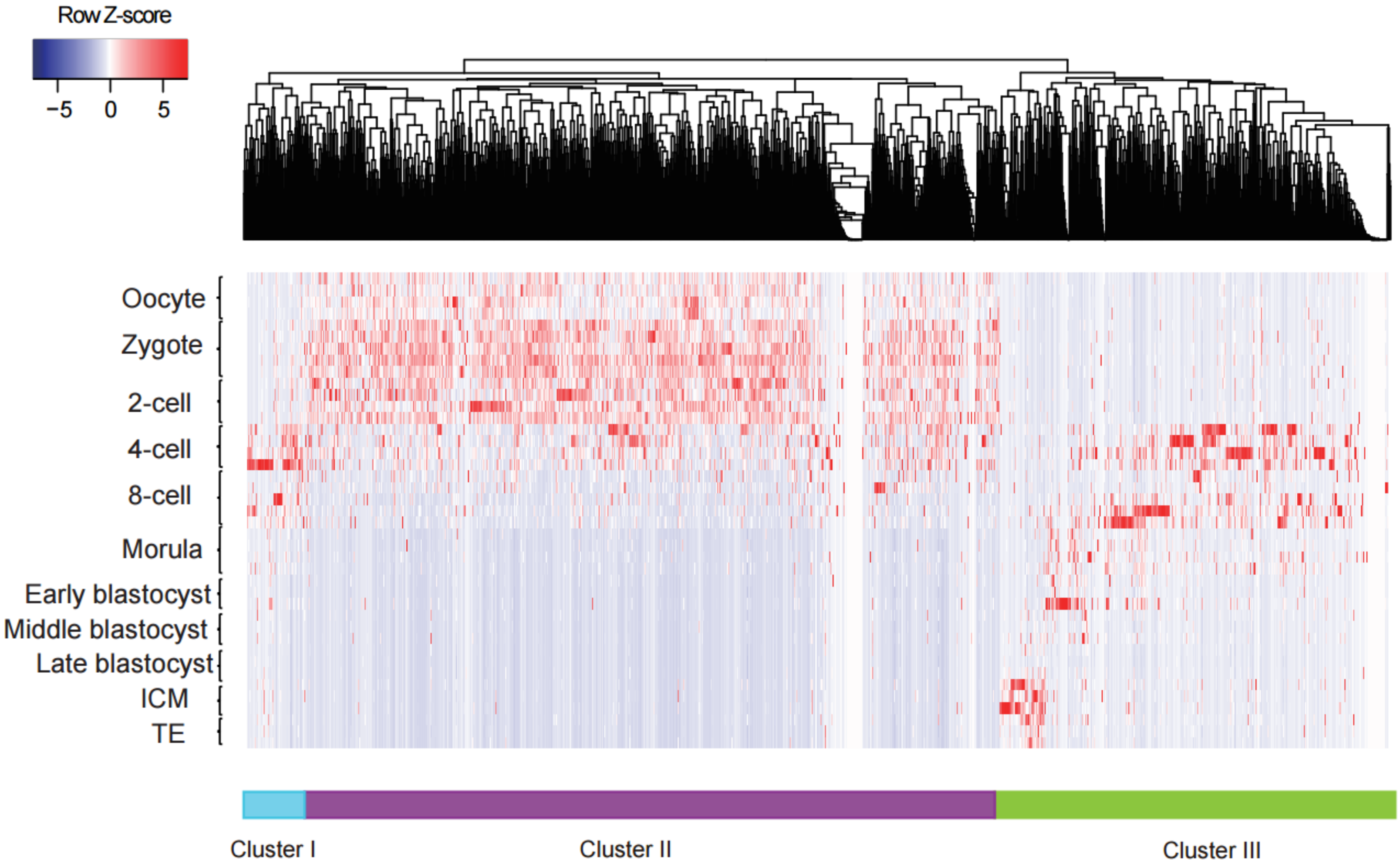

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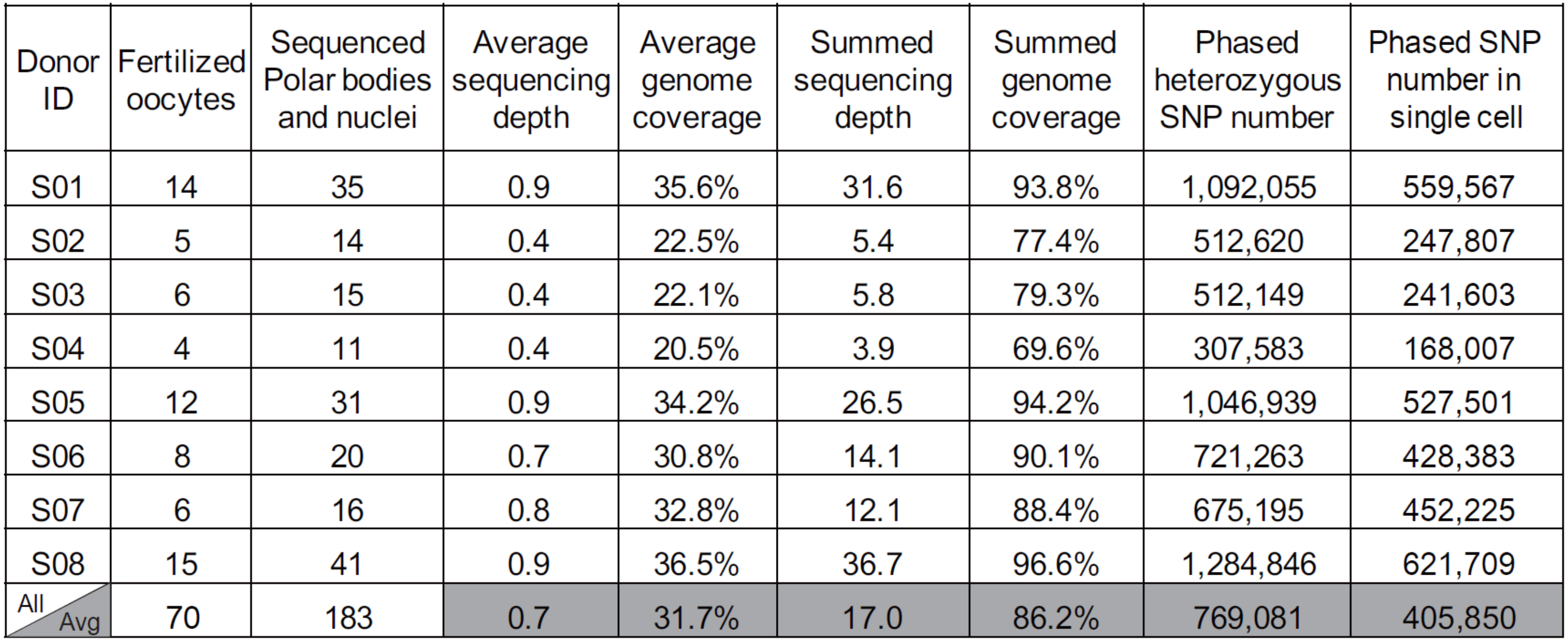
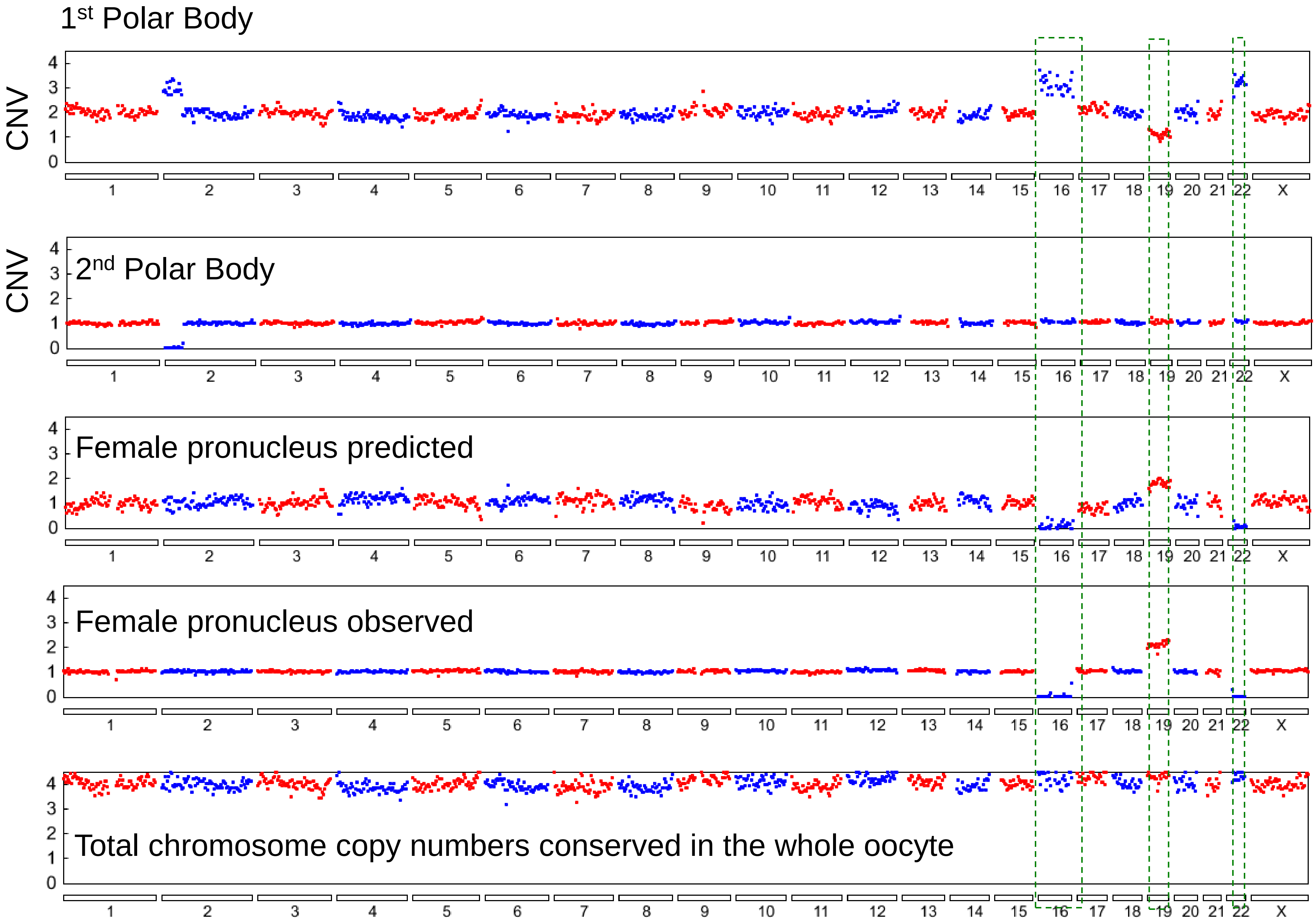


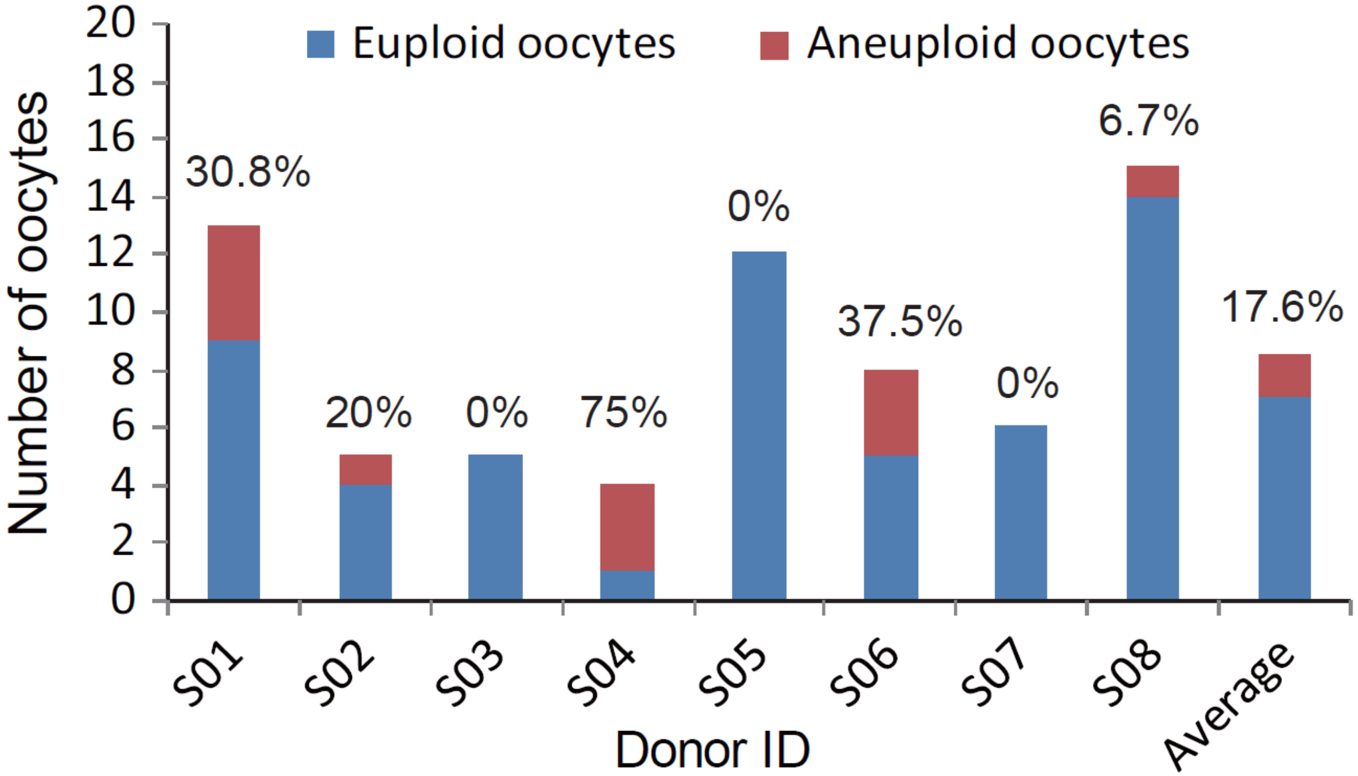
Donor ID	Fertilized oocytes	Sequenced Polar bodies and nuclei	Average sequencing depth	Average genome coverage	Summed sequencing depth	Summed genome coverage	Phased heterozygous SNP number	Phased SNP number in single cell
S01	14	35	0.9	35.6%	31.6	93.8%	1,092,055	559,567
S02	5	14	0.4	22.5%	5.4	77.4%	512,620	247,807
S03	6	15	0.4	22.1%	5.8	79.3%	512,149	241,603
S04	4	11	0.4	20.5%	3.9	69.6%	307,583	168,007
S05	12	31	0.9	34.2%	26.5	94.2%	1,046,939	527,501
S06	8	20	0.7	30.8%	14.1	90.1%	721,263	428,383
S07	6	16	0.8	32.8%	12.1	88.4%	675,195	452,225
S08	15	41	0.9	36.5%	36.7	96.6%	1,284,846	621,709
All Avg	70	183	0.7	31.7%	17.0	86.2%	769,081	405,850

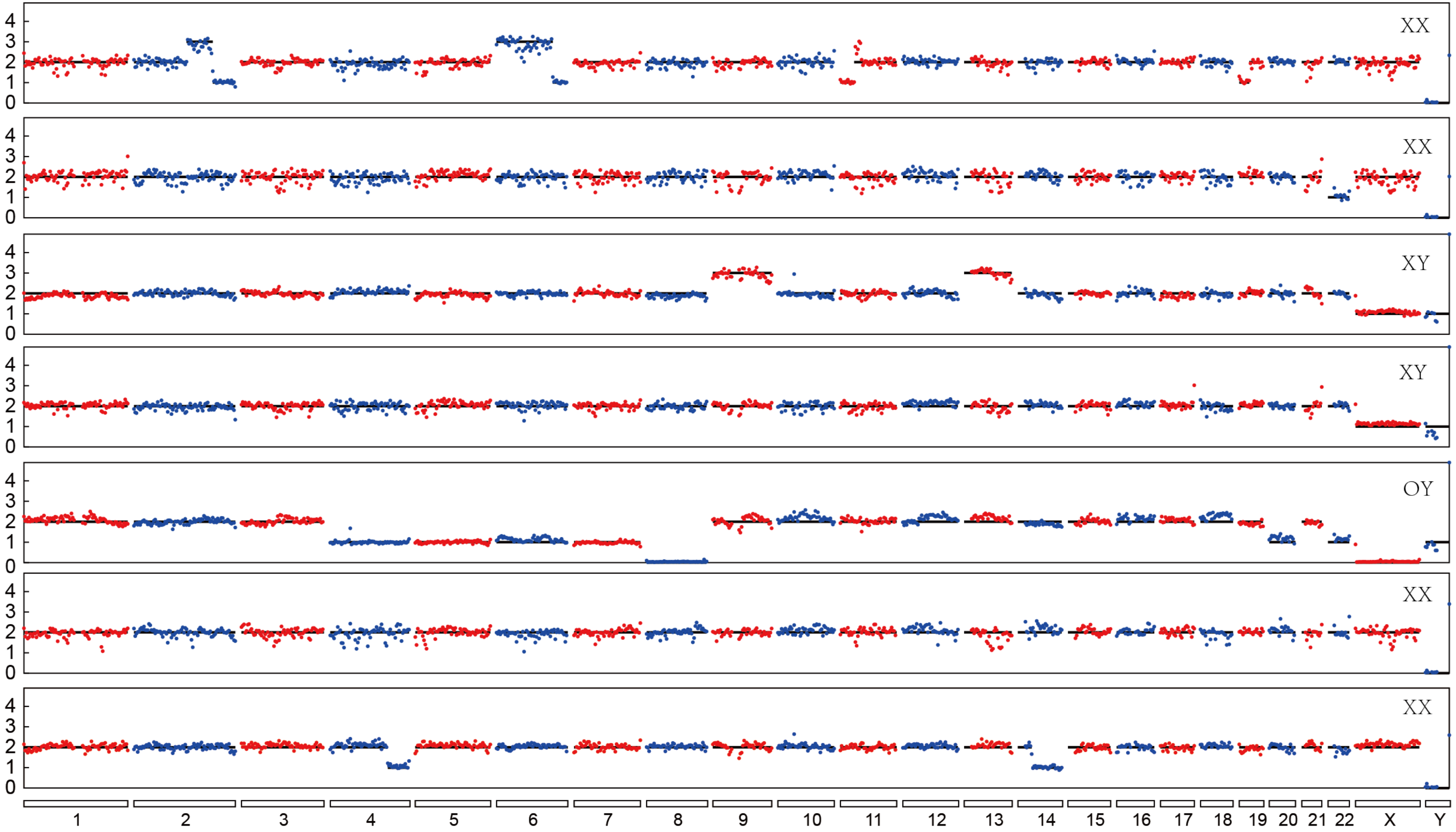
Summary of single cell genome sequencing of human oocytes
(Hou et al., Cell, 2013)

An Oocyte from Donor #1 with Chromosome Abnormality



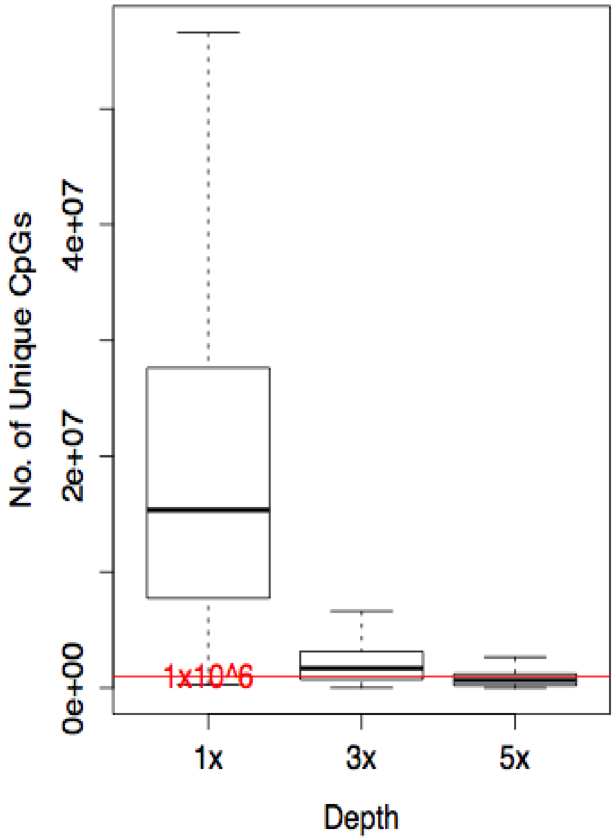
Aneuploidy rate for each donor





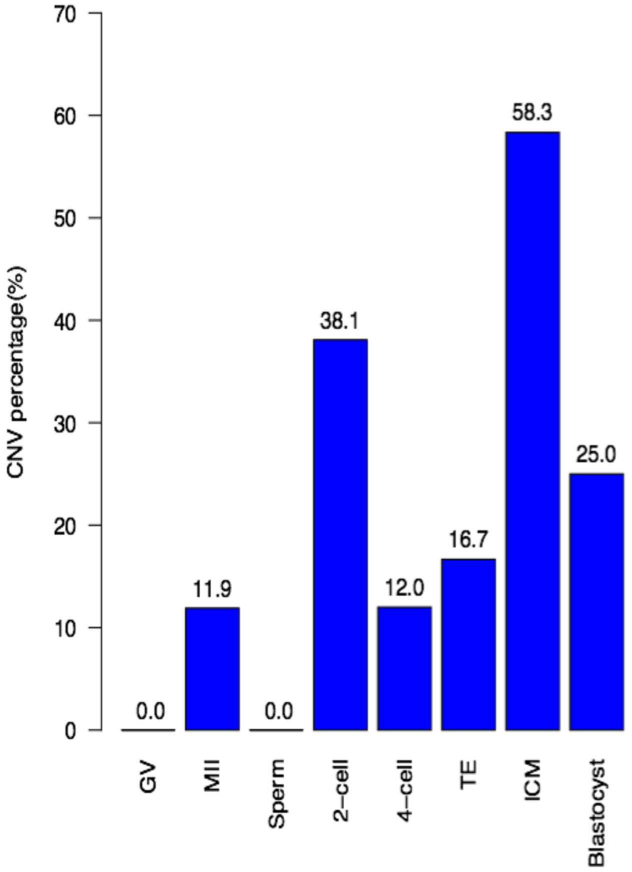
8-cell stage embryos from Donor #9 with Chromosome Abnormality

Stage	No. of Single Cells
GV oocyte	8
MII oocyte	42
Sperm	7
2-cell embryo	21
4-cell embryo	25
8-cell embryo	8
TE	9
ICM	11
Total	131



Single cell multiple omics sequencing of human preimplantation embryos (Unpublished)

Stage	No. of Samples	No. of Samples Showing CNVs
GV oocyte	8	0
MII oocyte	42	5
Sperm	9	0
2-cell embryo	21	8
4-cell embryo	25	3
TE	6	1
ICM	12	7
Blastocyst	4	1



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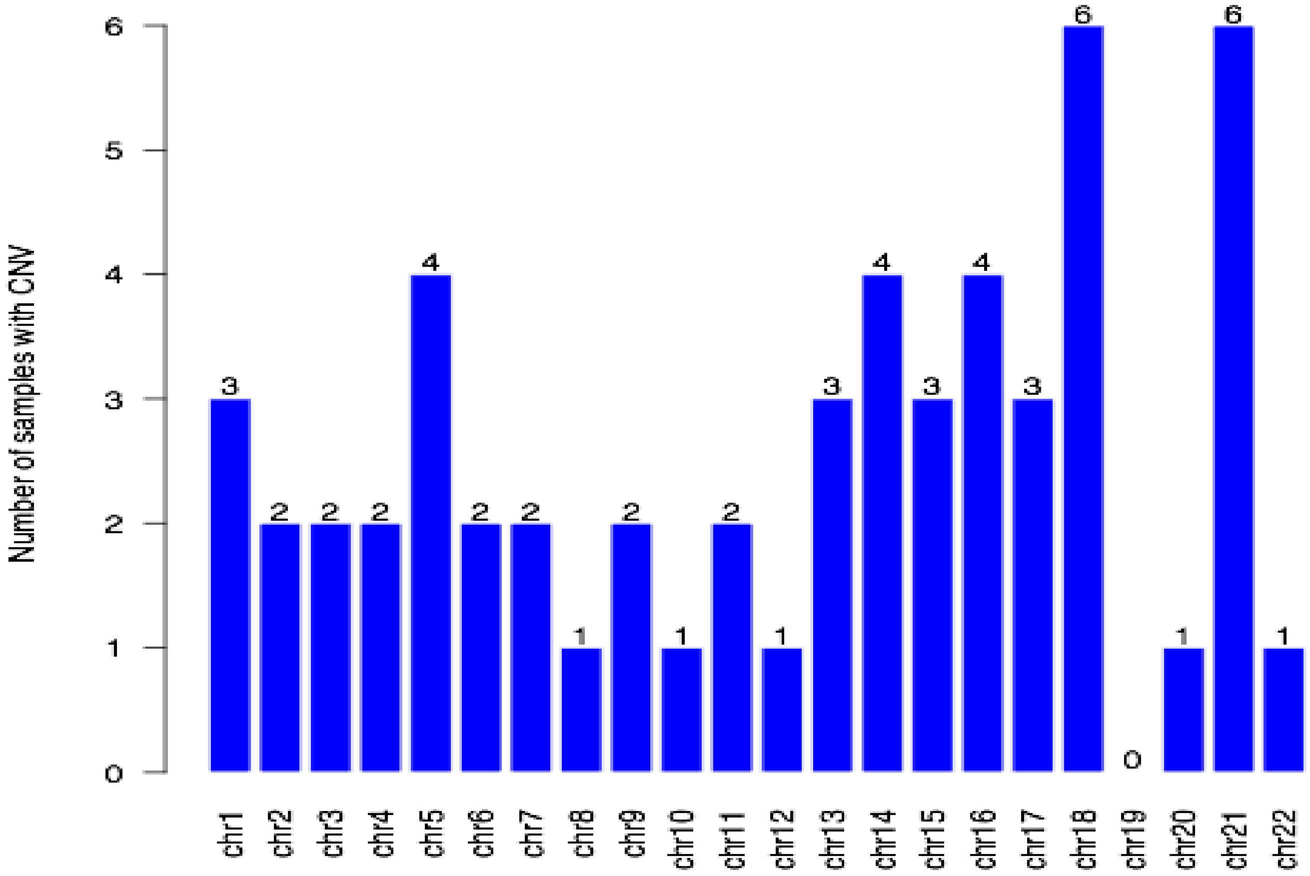
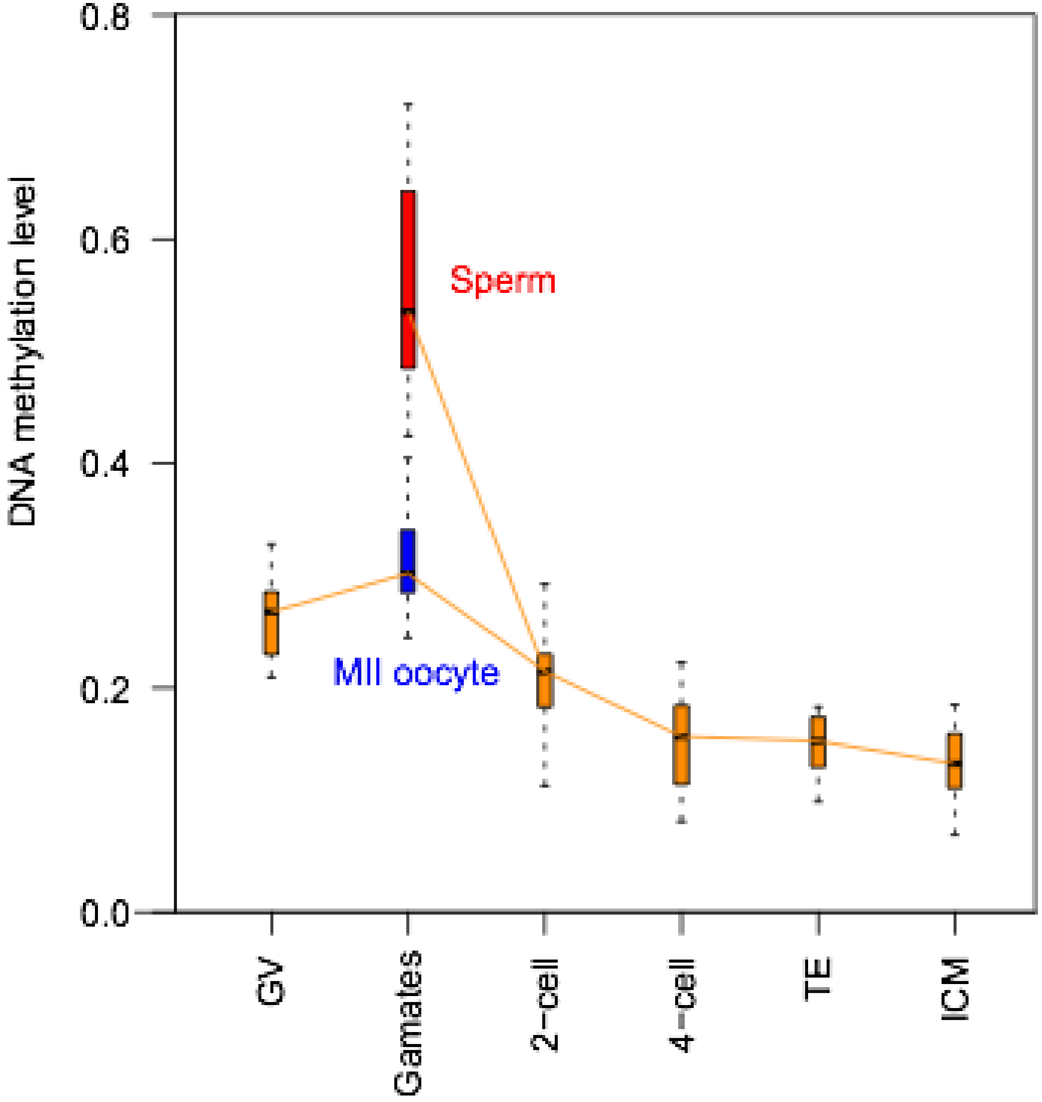


Single cell multiple omics sequencing of human preimplantation embryos (Unpublished)



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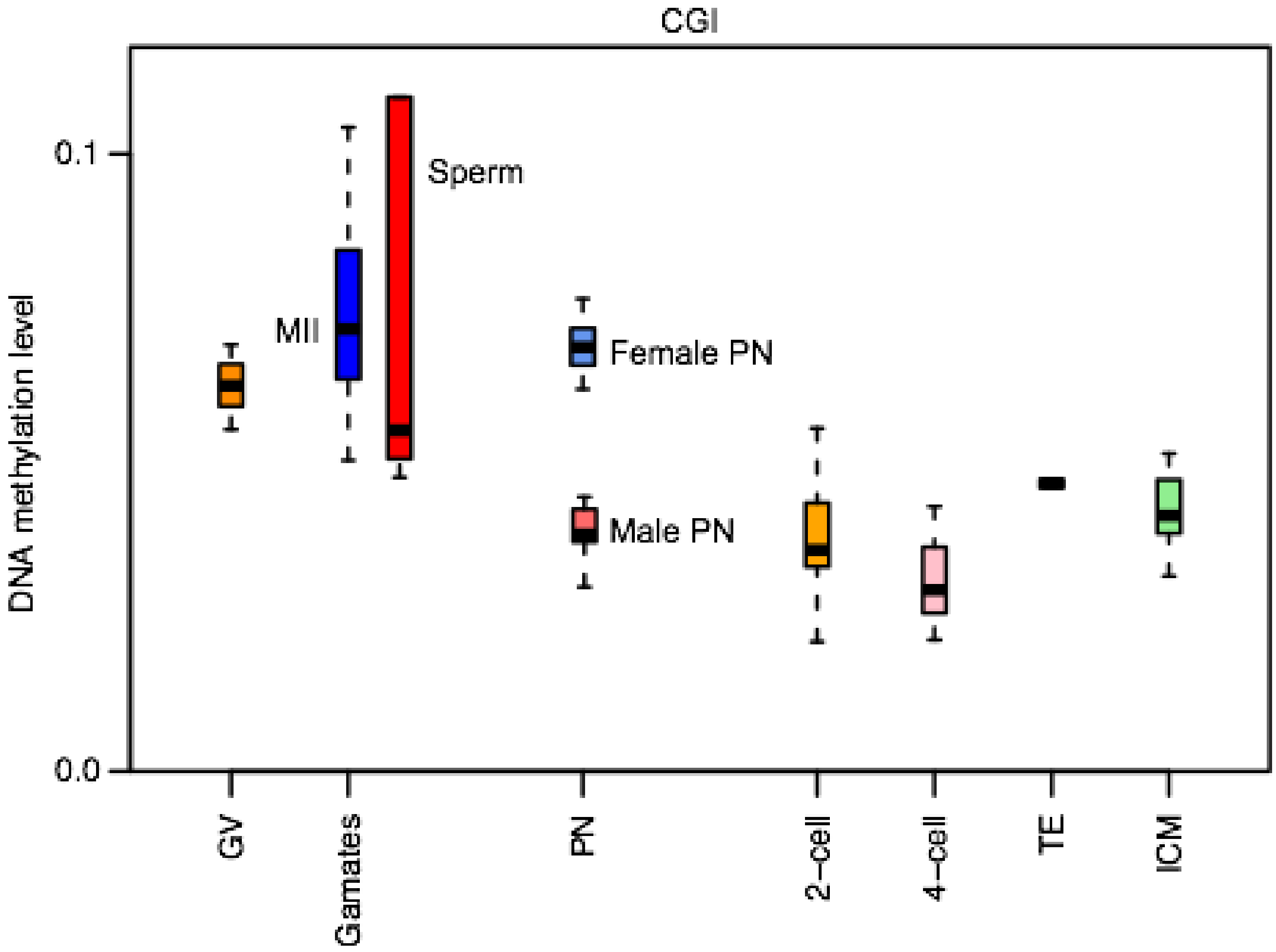

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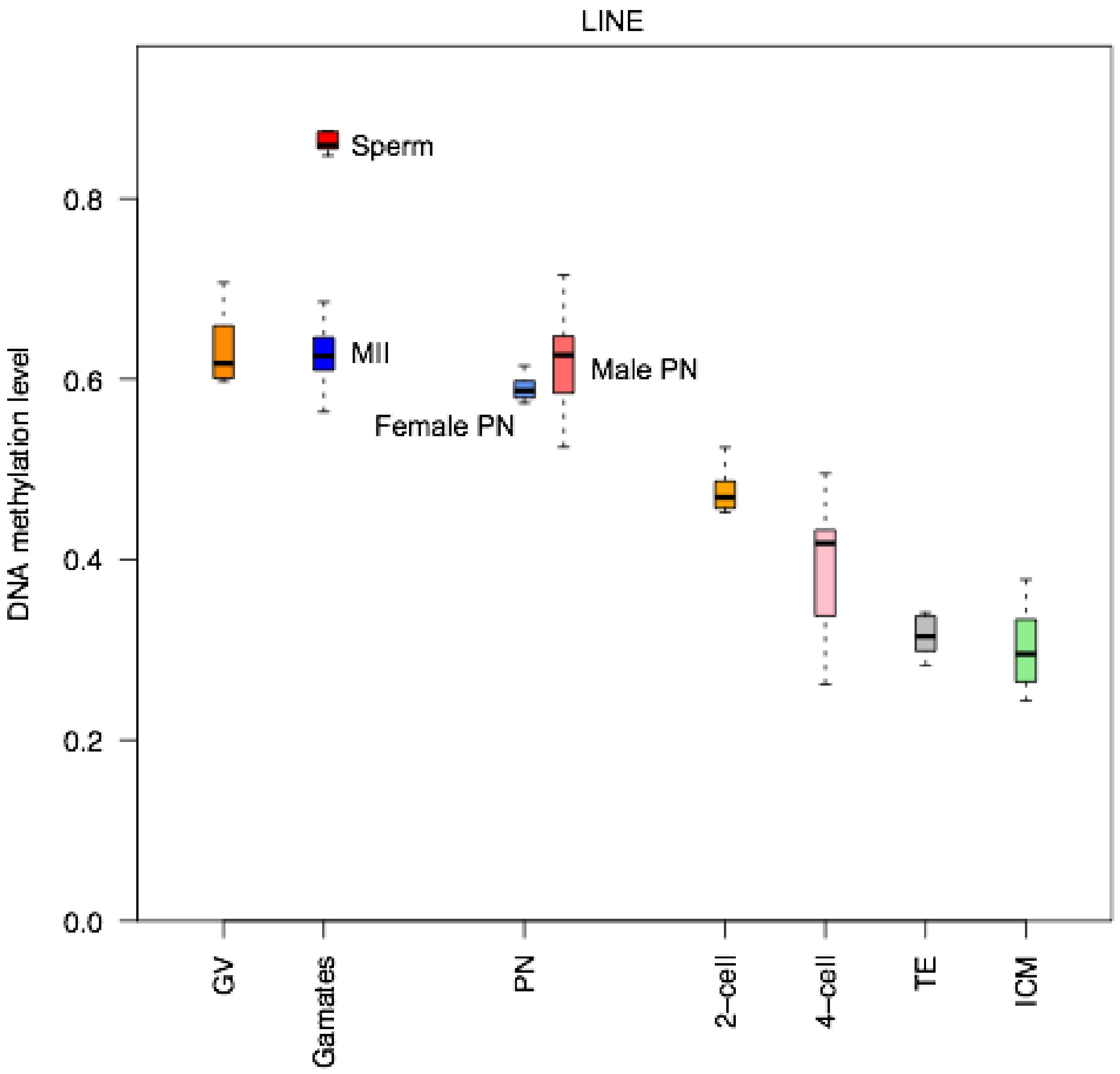

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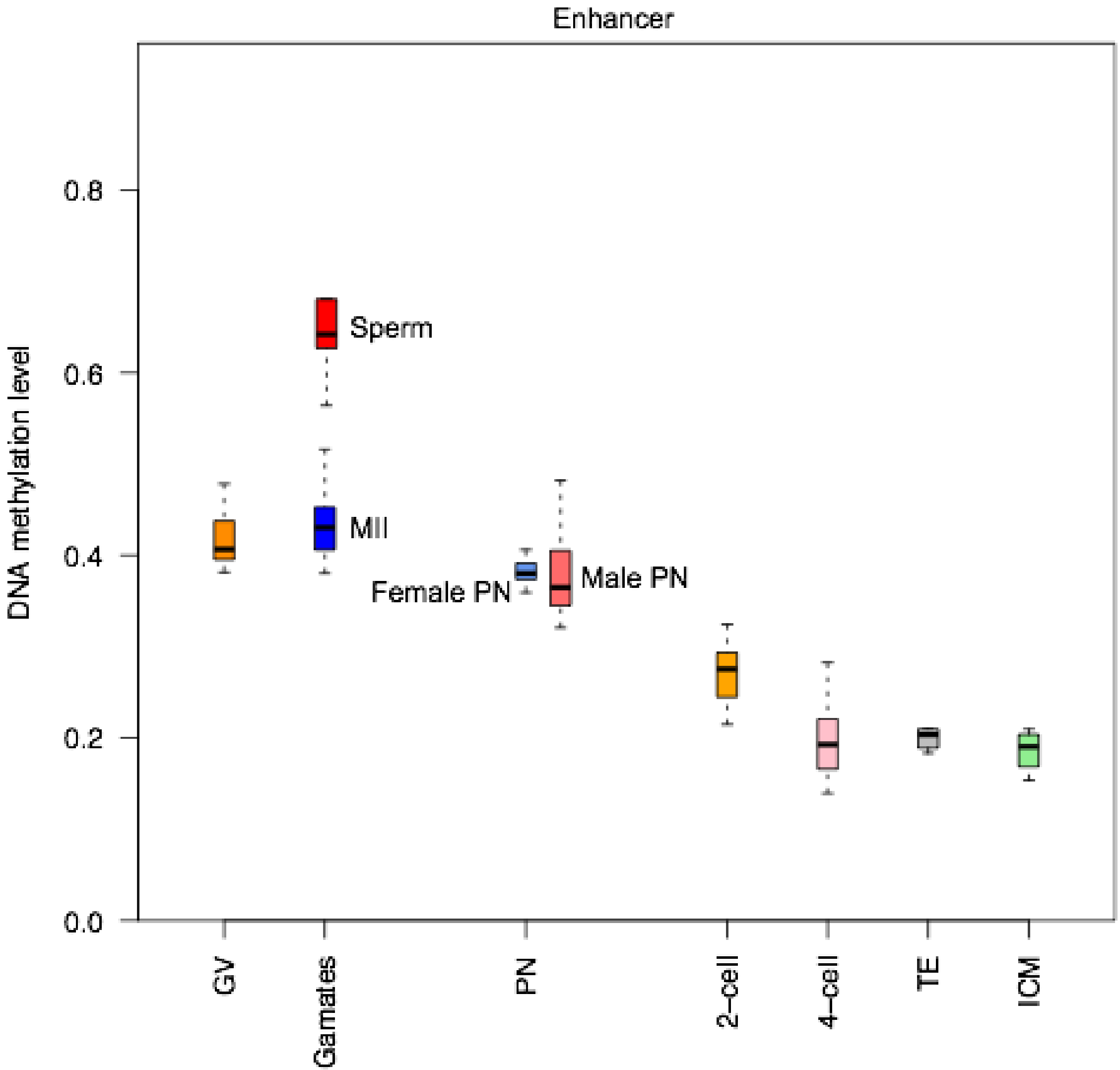

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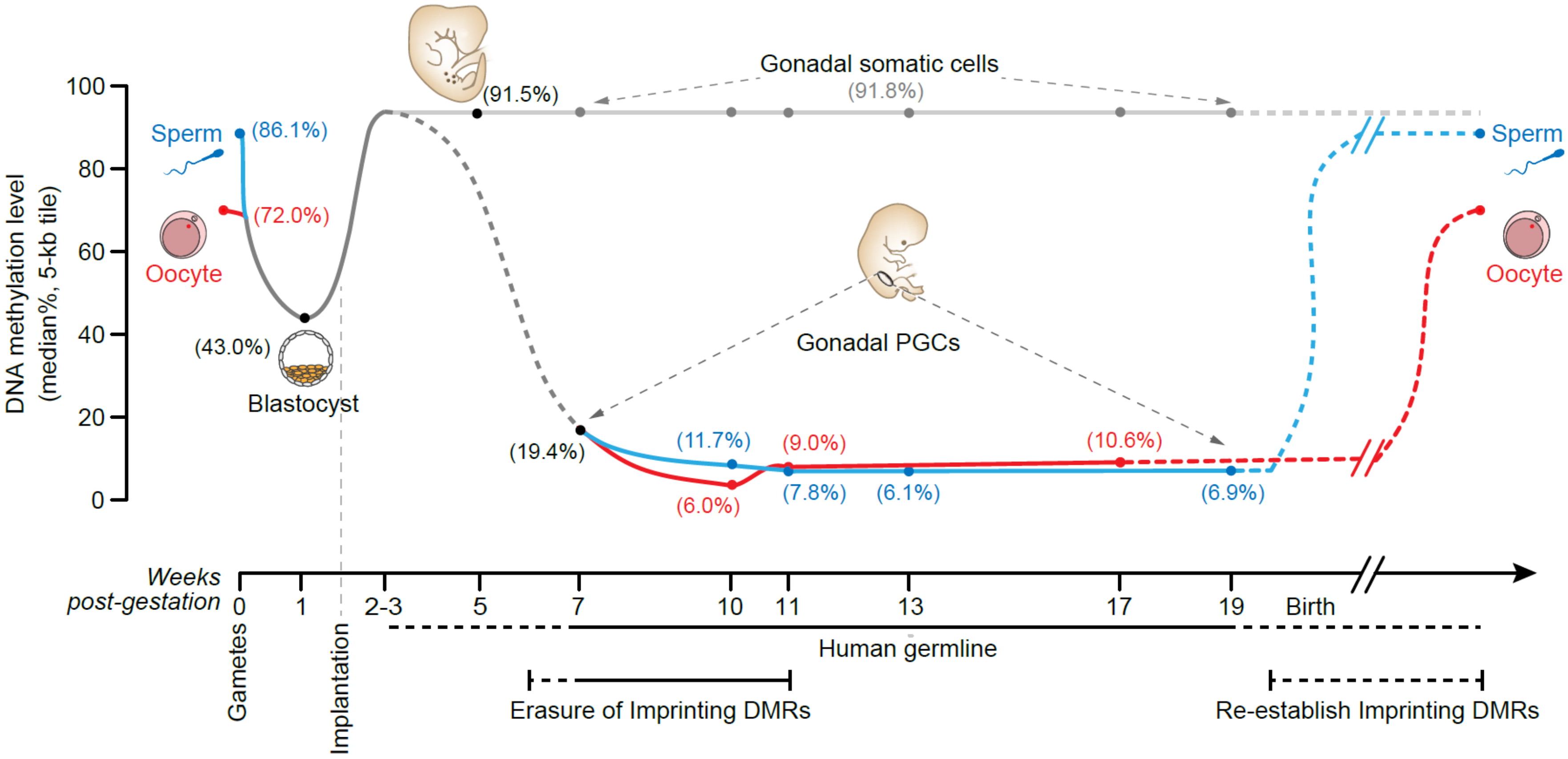

DNA methylation dynamics during human early embryonic development

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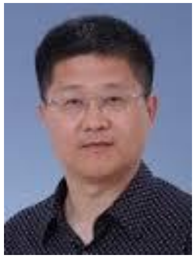
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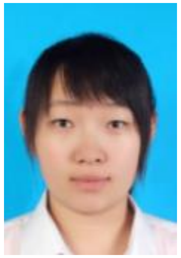
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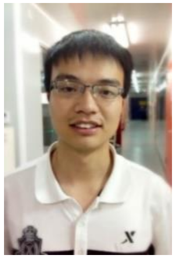
Hongshan Guo


Lin Li

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