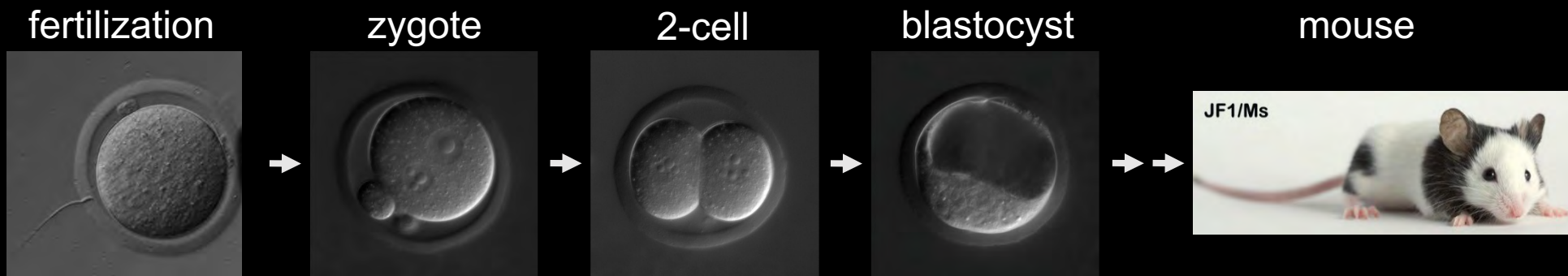


Cryptorchidism: considerations on mechanisms of epigenetic gene regulation and (intergenerational) epigenetic inheritance



CRYPTORCHIDISM: MOLECULAR BIOLOGY MEETS ENDOCRINOLOGY AND SURGERY

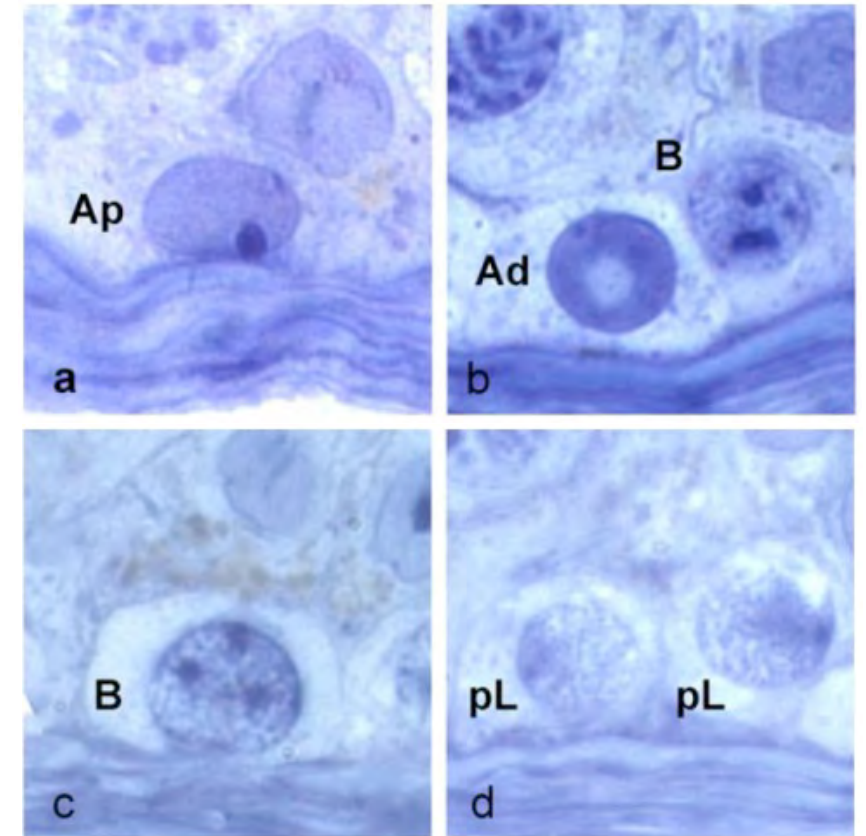
The 5th International Andrology Symposium,
International Conference Center, Valletta Malta
September 26 - 27, 2025

Gonadotropin-Releasing Hormone Agonist Corrects Defective Mini-Puberty in Boys with Cryptorchidism: A Prospective Randomized Study

The low fertility potential in men with surgically treated cryptorchidism is asserted to be hormonal in origin; it arises as a consequence of impaired “mini-puberty”, a period when gonocytes translate into adult (dark) spermatogonia [7]. In male infants, mini-puberty occurs between two and four months after birth, when an increase in gonadotropin secretion stimulates Leydig cells to secrete testosterone [8, 9].

This testosterone increase is blunted in infants with cryptorchidism [10–13], and it results in an **inadequate transition of gonocytes into adult dark (Ad) spermatogonia** [7, 14].

Vincel *et al.*, 2018



Muciaccia *et al.*, 2013

Gene Expression Changes Underlying Idiopathic Central Hypogonadism in Cryptorchidism with Defective Mini-Puberty

Faruk Hadziselimovic^a Katharina Gegenschatz-Schmid^a Gilvydas Verkauskas^e
Maria J. Docampo-Garcia^a Philippe Demougin^b Vytautas Bilius^e
Dalius Malcius^g Darius Dasevicius^f Michael B. Stadler^{c, d}

^aCryptorchidism Research Institute, Kindermedizinisches Zentrum Liestal, Liestal, and ^bBiozentrum, Life Sciences Training Facility, University of Basel, ^cFriedrich Miescher Institute for Biomedical Research, and ^dSwiss Institute of Bioinformatics, Basel, Switzerland; ^eChildren's Surgery Centre, Vilnius University, and ^fInstitute for Pathology, National Centre of Pathology, Vilnius, and ^gLithuanian University of Health Sciences, Kaunas, Lithuania

Many genes are less highly expressed in gonads of HIR patients compared to LIR patientsCV

HIR: high infertility risk patients – absence of A-dark spermatogonia
LIR: low infertility risk patients – presence of A-dark spermatogonia

d Heatmap showing the log2 expression of the 1,000 most significant differential genes between HIR and LIR patients relative to the average expression of each gene across all patients. Patient age and group are indicated by color on top of the heatmap, and both patients and genes have been reordered by hierarchical clustering to group similar profiles (clustering dendrograms shown on top and on the left side).

... reflecting a failure to change cell identity from gonocytes to spermatogonia?

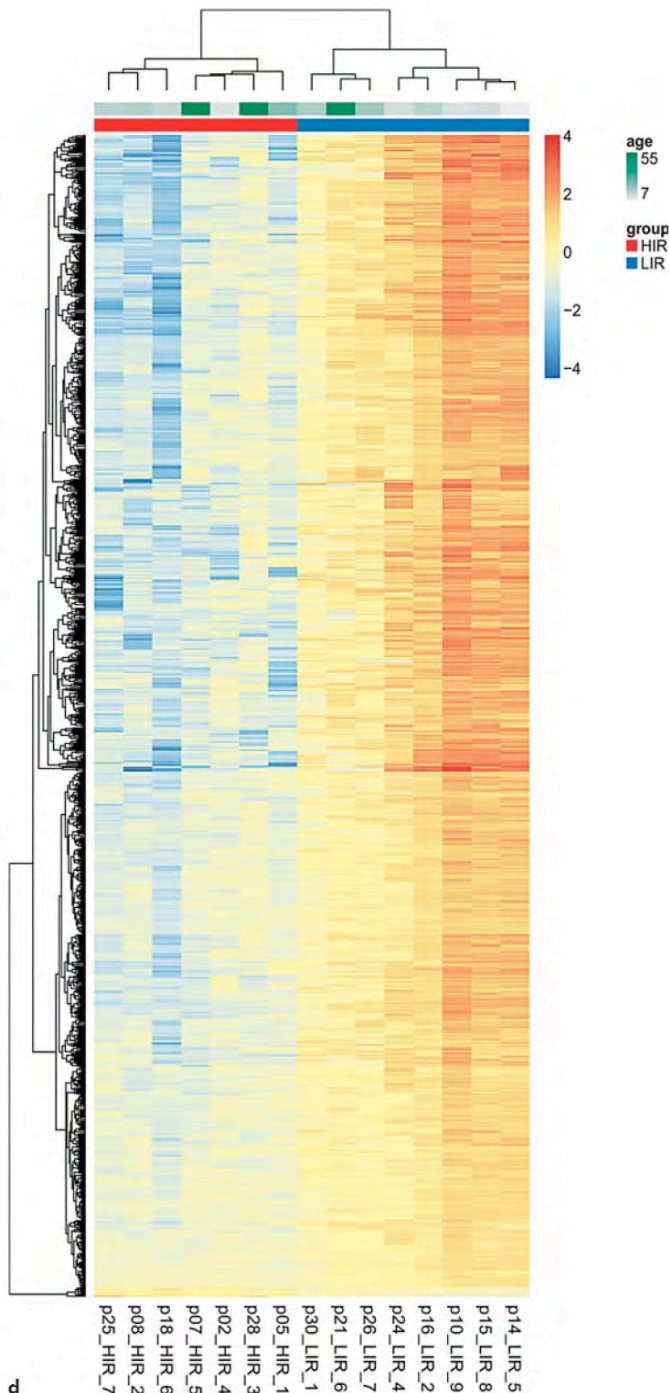


Table 2. New identified genes downregulated in HIR and involved in the HPG axis

Gene ID	Name	HIR median	LIR median	logFC ^a	FDR
<i>ARID3B</i>	AT rich interactive domain 3B (BRIGHT-like)	1.25	3.35	-1.44965	0.000171
<i>ATF3</i>	activating transcription factor 3	0.47	1.10	-0.96099	0.009544
<i>AXIN2</i>	axin 2	2.33	5.75	-1.28981	0.000283
<i>BMP7</i>	bone morphogenetic protein 7	0.27	0.76	-1.58963	0.00144
<i>BMPR1B</i>	bone morphogenetic protein receptor, type IB	0.72	3.90	-2.5653	0.000184
<i>CHD7</i>	chromodomain helicase DNA binding protein 7	3.57	5.94	-0.72742	0.001522
<i>CLOCK</i>	clock circadian regulator	9.47	13.82	-0.46419	0.01024
<i>DLX2</i>	distal-less homeobox 2	0.05	0.28	-2.60142	0.003894
<i>DLX3</i>	distal-less homeobox 3	0.06	0.28	-2.07726	0.006612
<i>DLX5</i>	distal-less homeobox 5	0.20	0.87	-1.92109	0.00407
<i>DUSP4</i>	dual specificity phosphatase 4	0.21	0.74	-1.73444	0.000154
<i>EBF3</i>	early B-cell factor 3	0.33	1.59	-1.94656	0.000218
<i>EGR2</i>	early growth response 2	0.34	0.84	-1.17858	0.001333
<i>FGF14</i>	fibroblast growth factor 14	0.19	0.49	-1.23942	0.020814
<i>FGFR1</i>	fibroblast growth factor receptor 1	4.79	7.61	-0.49372	0.024252
<i>FGFR2</i>	fibroblast growth factor receptor 2	1.98	4.32	-1.25292	0.00052
<i>FGFRL1</i>	fibroblast growth factor receptor-like 1	1.33	2.84	-0.97919	0.010591
<i>GATA2</i>	GATA binding protein 2	0.33	1.06	-1.52714	0.000339
<i>ISL2</i>	ISL LIM homeobox 2	0.08	0.27	-1.6183	0.008855
<i>KLF4</i>	Kruppel-like factor 4 (gut)	1.43	4.11	-1.16046	0.008282
<i>LHX2</i>	LIM homeobox 2	0.03	0.28	-3.13022	0.0019
<i>LHX6</i>	LIM homeobox 6	0.35	1.17	-1.72084	0.000238
<i>LIN28B</i>	lin-28 homolog B (<i>C. elegans</i>)	0.43	1.78	-2.2908	0.000264
<i>MSX1</i>	msh homeobox 1	0.29	0.69	-1.37378	0.00227
<i>NHLH2</i>	nescient helix loop helix 2	0.21	0.62	-1.65737	0.000772
<i>NOS1</i>	nitric oxide synthase 1 (neuronal)	0.12	0.29	-1.57741	0.000352
<i>NR4A1</i>	nuclear receptor subfamily 4, group A, member 1	1.52	2.80	-1.05698	0.00366
<i>OTX2</i>	orthodenticle homeobox 2	0.12	0.34	-1.7389	0.03221
<i>PCSK1N</i>	proprotein convertase subtilisin/kexin type 1 inhibitor	0.28	1.87	-2.57264	0.003657
<i>PITX1</i>	paired-like homeodomain 1	0.00	0.26	-4.21104	0.000866
<i>PITX2</i>	paired-like homeodomain 2	0.07	0.47	-2.36513	0.00097
<i>POU3F1</i>	POU class 3 homeobox 1	0.09	0.43	-2.53507	0.000391
<i>PROK2</i>	prokineticin 2	0.22	1.05	-2.43563	0.000184
<i>RUNX1</i>	runt-related transcription factor 1	0.40	0.67	-0.95564	0.008482
<i>SEMA3F</i>	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F	0.15	0.43	-1.67332	0.004377
<i>SEMA4D</i>	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D	2.27	4.07	-0.83474	0.001076
<i>SPRY4</i>	sprouty homolog 4 (<i>Drosophila</i>)	0.46	1.14	-1.05327	0.001517
<i>TRPC3</i>	transient receptor potential cation channel, subfamily C, member 3	0.45	1.04	-1.34278	0.004737

^a Absolute fold-changes <2 are highlighted in italics.

Failed upregulation of novel genes involved in the HPG axis in gonadal samples of high infertility risk patients.

Absence of HPG-mediated activation of genes in gonocytes?

Intrinsically impaired activation and/or aberrant repression during differentiation towards spermatogonial fate?

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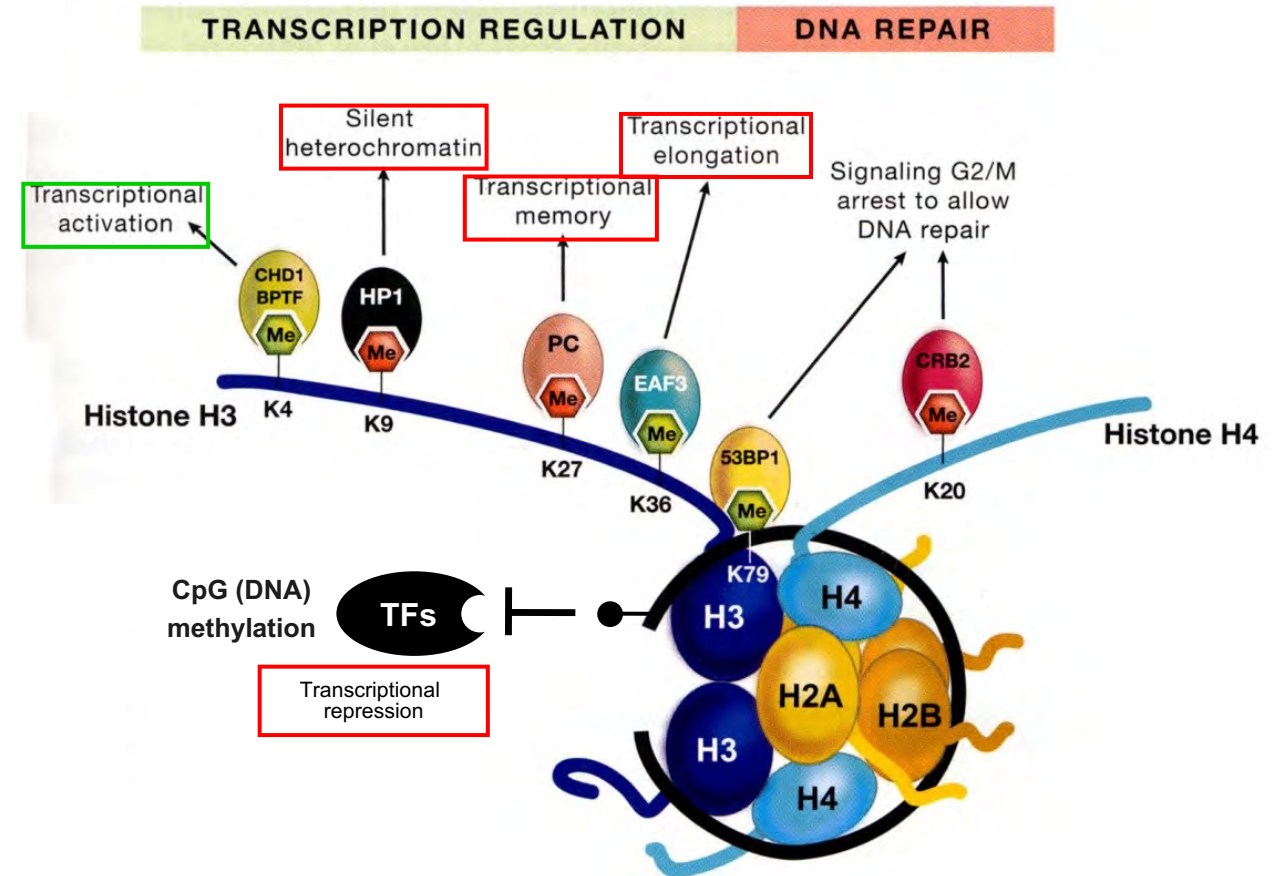
Role of transcription factors and/or chromatin?

Modes of epigenetic gene regulation and inheritance

Chromatin organization



Nucleosomes and modifications



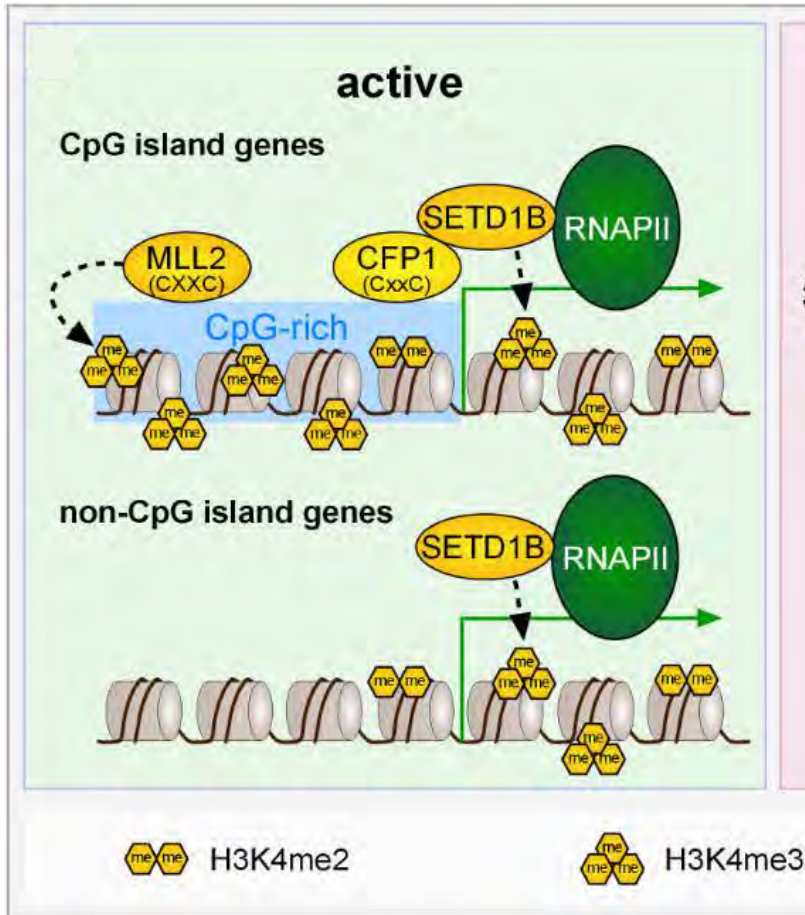
Major modes of chromatin-related inheritance

1. DNA methylation ($\rightarrow$ more recent focus)
2. Nucleosomes with PTMs ($\rightarrow$ long standing focus)
3. RNA (mRNA, ncRNA, small RNAs, tRNAs, rRNAs)

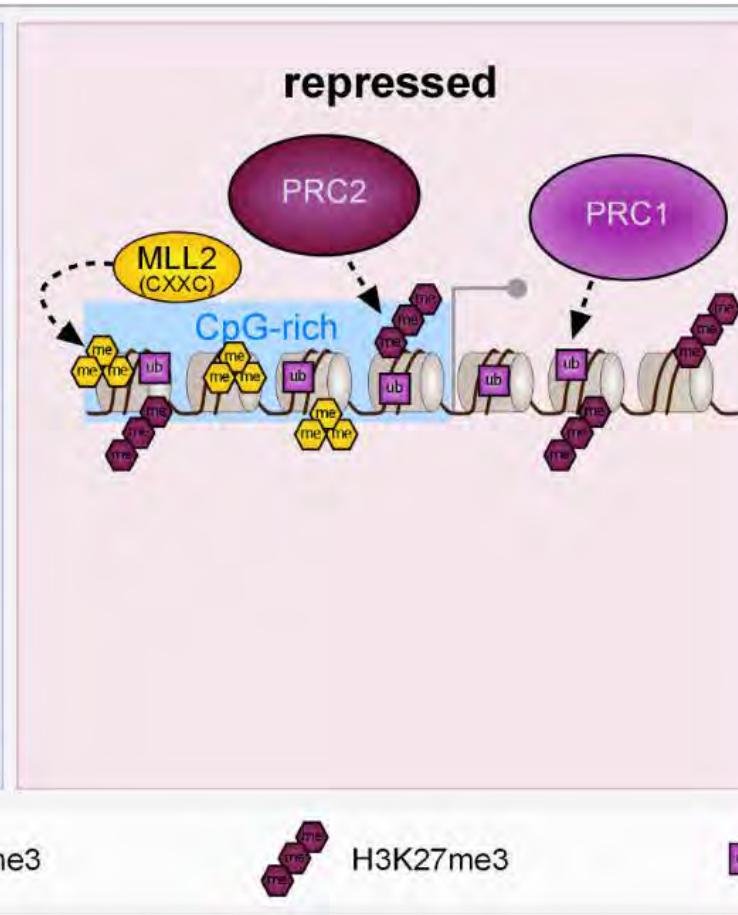
Instructive and antagonistic interplay

Role of histone and DNA modifications in gene regulation

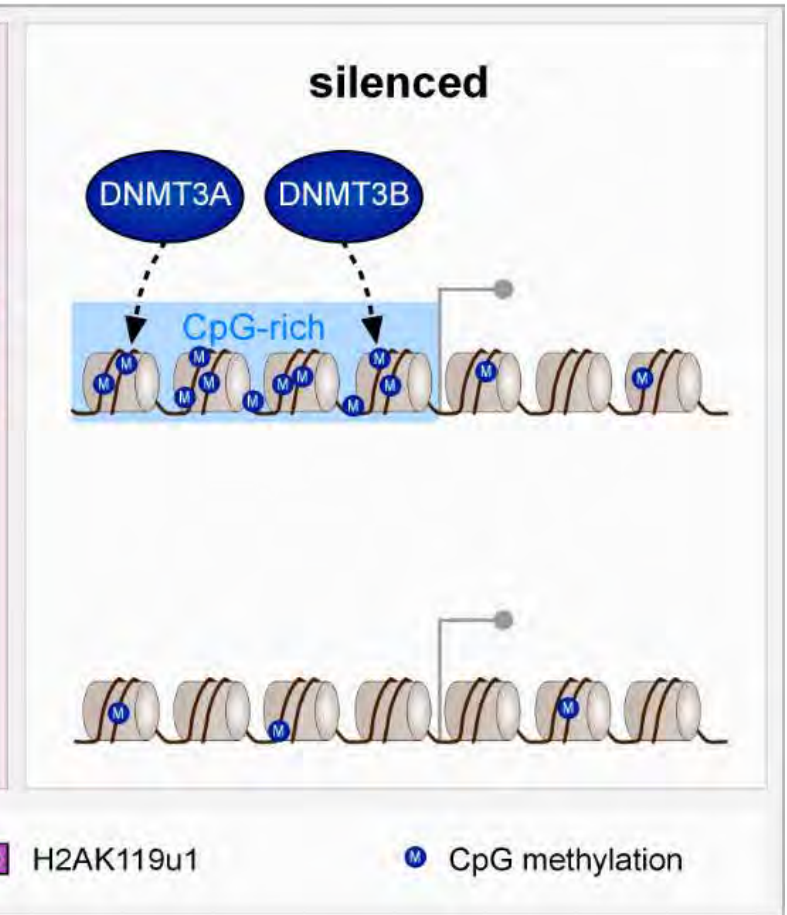
H3K4me3 activity



Polycomb repression



DNA methylation



housekeeping and developmental genes

developmental regulatory genes

germline genes

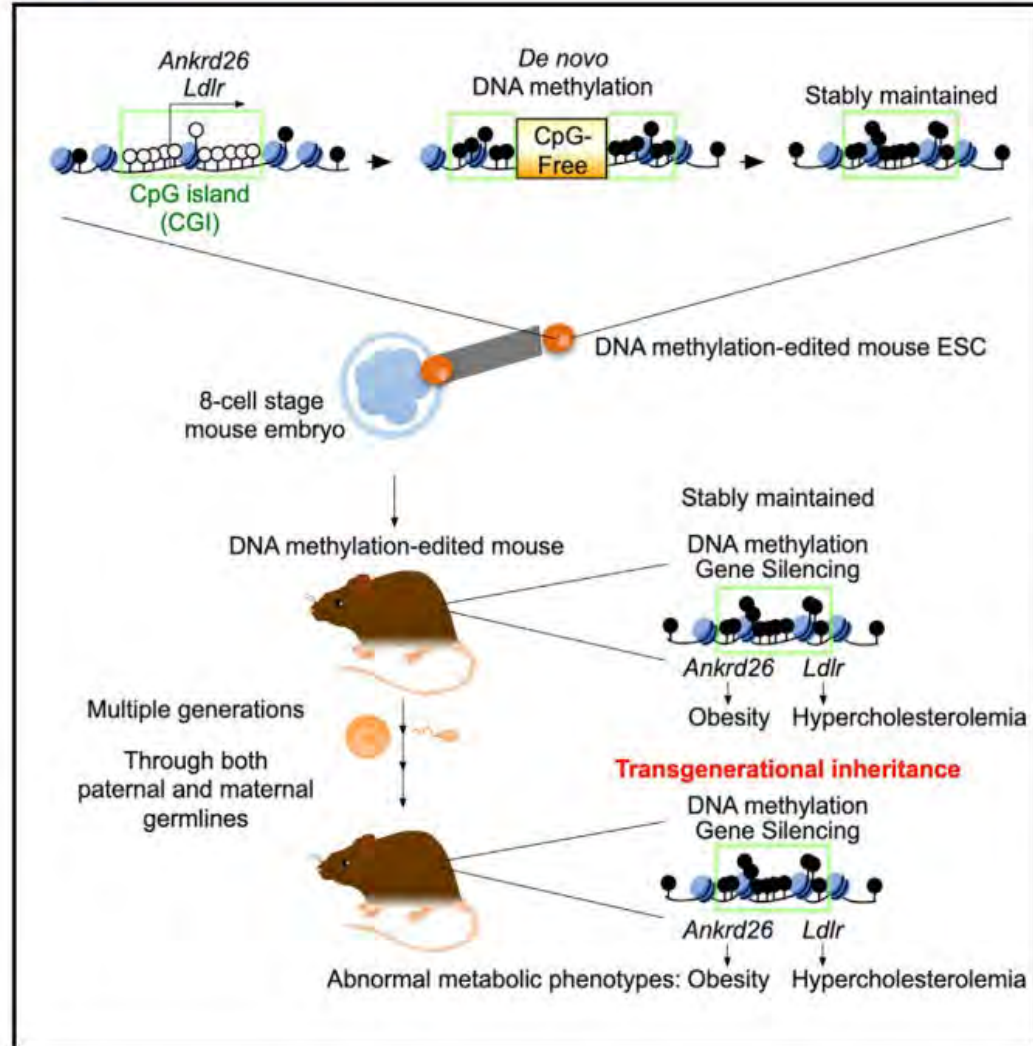
RESEARCH ARTICLE

Abnormal H3K27me3 underlies degenerative spermatogonial stem cells in cryptorchid testis

Kazushige Kuroha*, Ivana Dočkal*, Uroš Radović, Kuniko Nakajima, Ikue Hoshi, Shion Matsuda, Noriko Kojitani, Kazuyuki Ohbo and Shin-ichi Tomizawa[‡]

Transgenerational inheritance of acquired epigenetic signatures at CpG islands in mice

Graphical abstract



Authors

Yuta Takahashi,
Mariana Morales Valencia, Yang Yu, ...,
Estrella Nuñez-Delicado,
Concepcion Rodriguez Esteban,
Juan Carlos Izpisua Belmonte

Correspondence

jcbelmonte@altoslabs.com

In brief

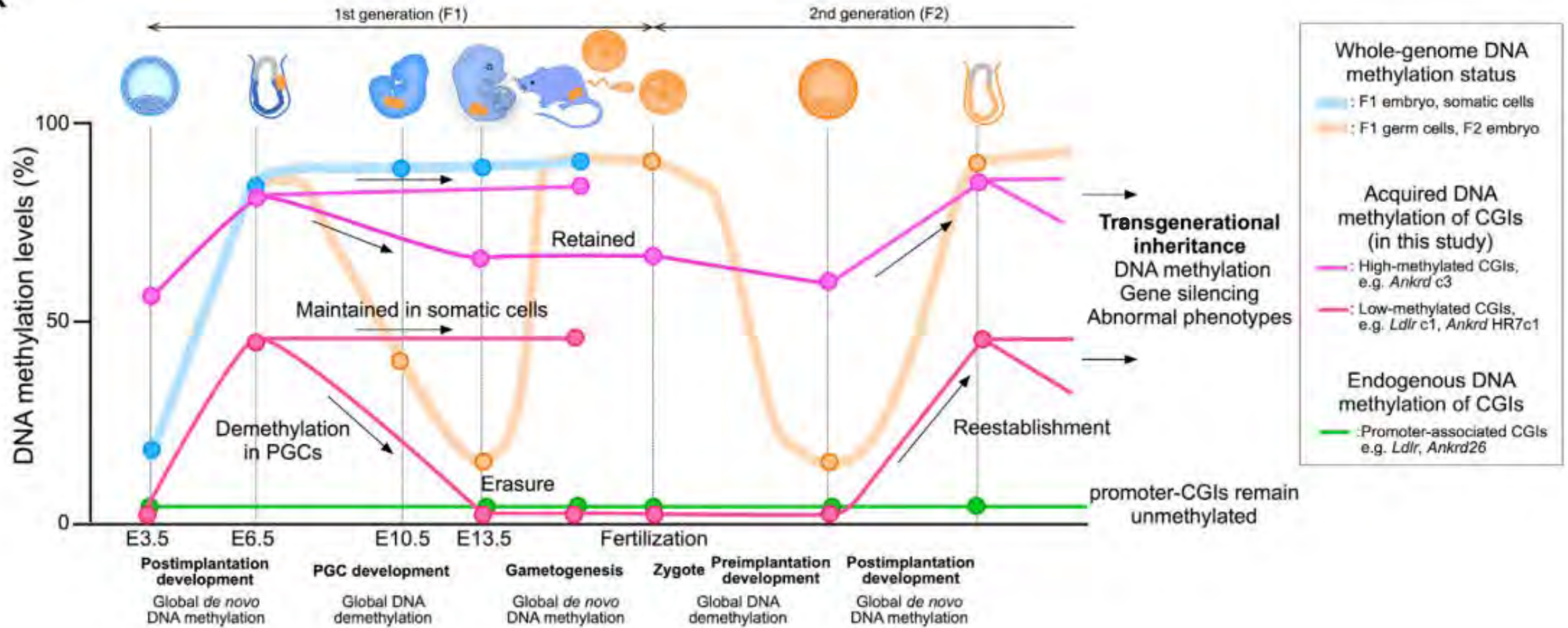
Takahashi et al. demonstrate that engineered epigenetic modifications and associated phenotypes can be transmitted across multiple generations in mammals.

Highlights

- Establishment of targeted CGI methylation editing in mouse ES cells
- DNA methylation-edited mice exhibit phenotypic alterations induced by gene silencing
- Both acquired methylation and phenotypes are transmitted across multiple generations
- Heritable epigenetic memory reestablishes CGI methylation at the epiblast stage

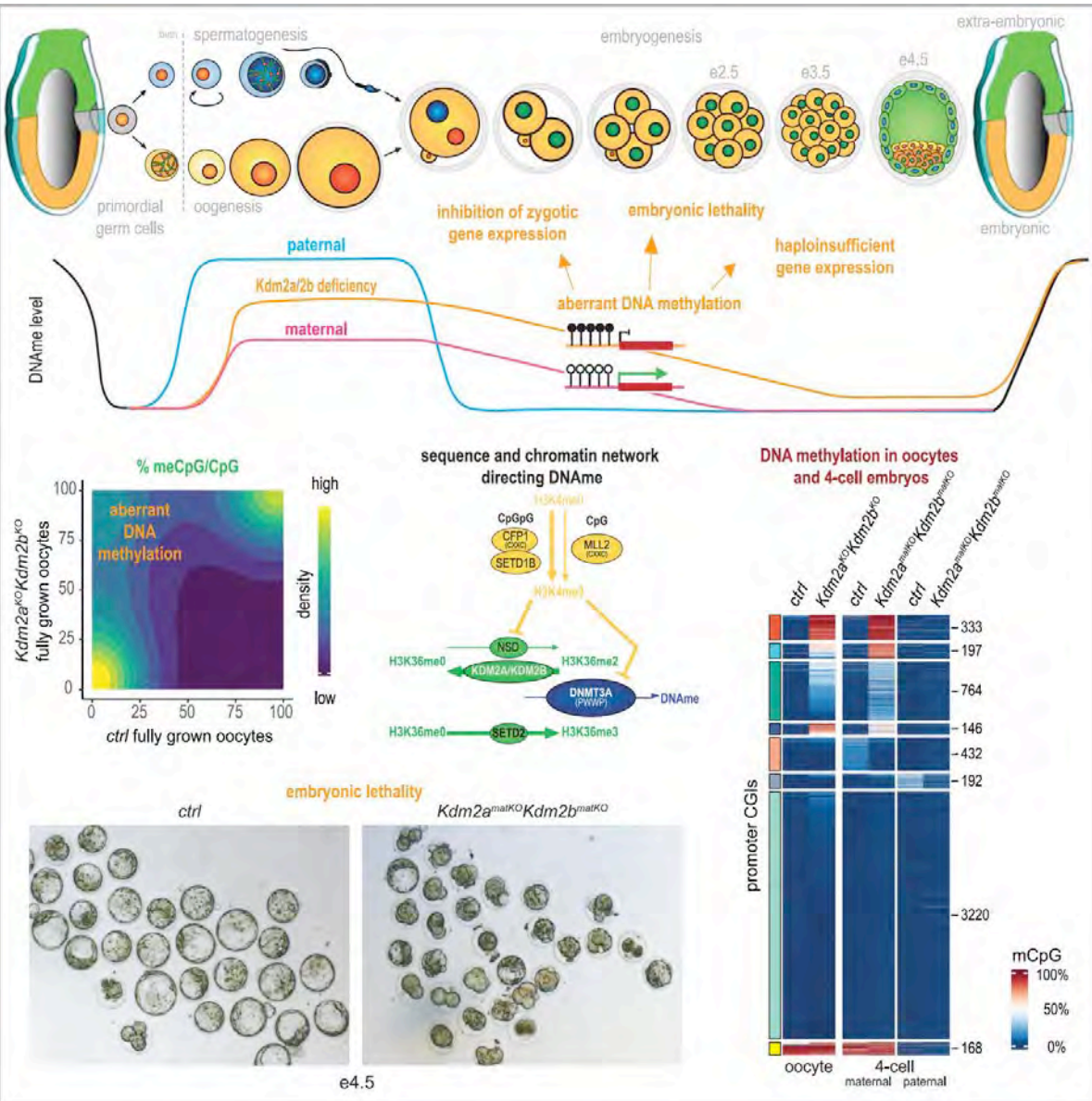
Acquired DNA methylation is only in part maintained in PGCs, and during gametogenesis and pre-implantation development, prior to its reestablishment during post-implantation development

K



Developmental Cell

Preventing CpG hypermethylation in oocytes safeguards mouse development



Authors

Yumiko K. Kawamura,
Evgeniy A. Ozonov,
Panagiotis Papasaikas, ...,
Sebastien A. Smallwood,
Haruhiko Koseki, Antoine H.F.
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In brief

Kawamura et al. show that H3K36me2 removal by the KDM2A and KDM2B histone demethylases restricts DNMT3A in establishing global DNA methylation in oocytes. Maternal *Kdm2a/Kdm2b* knockout embryos die during pre-implantation development with impaired gene transcription, identifying limited DNA methylation reprogramming and susceptibility to gene dosage haplo-insufficiency effects in early mouse embryos.

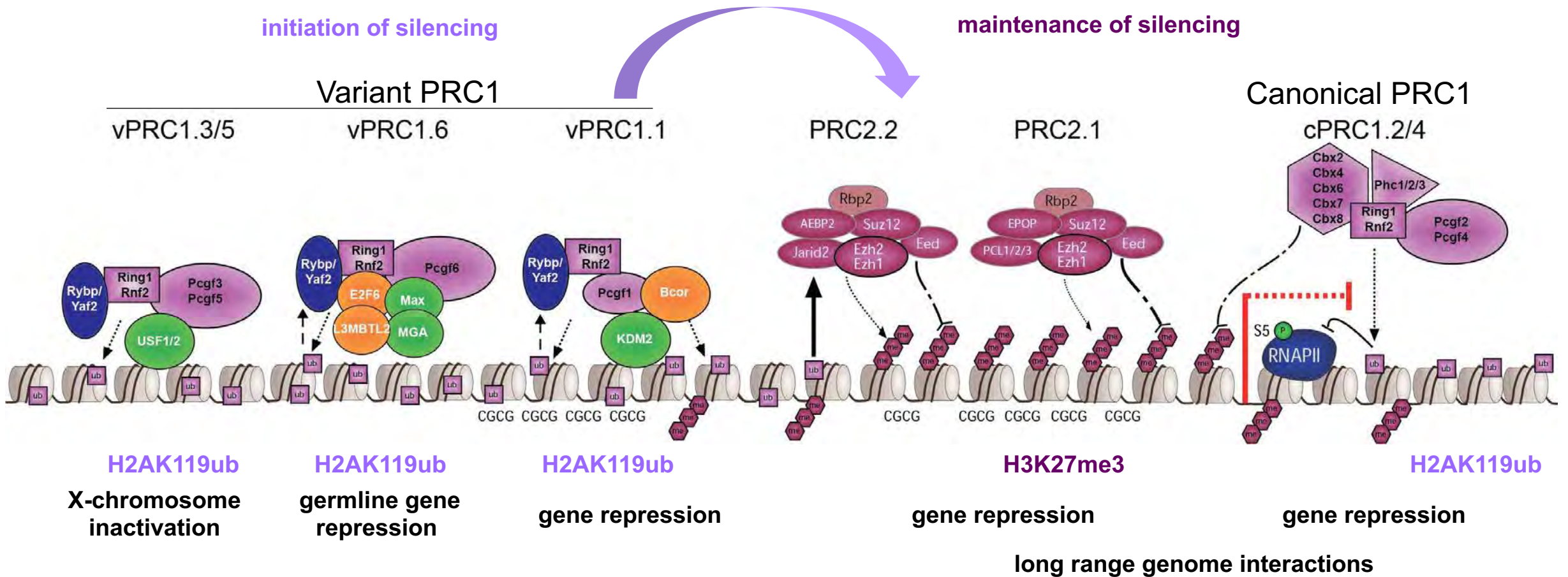
Highlights

- KDM2A/KDM2B prevent *de novo* DNA methylation in mouse oocytes by demethylating H3K36me2
- DNA sequence and H3K4me3 control aberrant H3K36me2 and DNAm acquisition at CGIs
- Aberrant oocyte DNAm is not reprogrammed in early embryos and represses transcription
- Intergenerational inheritance of aberrant oocyte DNAm is lethal for early embryos

**Michael T. Bocker^{1,9}, Grigorios Fanourgakis ^{2,9}, Kristie Wetzel³, Pavel A. Komarov ^{2,4}, H  l  ne Royo^{2,5}, Alexia Rohmer², Sunwoo Chun^{2,4,6}, Ching-Yeu Liang^{2,4}, Hubertus Kohler², Taiping Chen ^{1,7}, Xiaohong Mao³, Mark A. Labow¹, Reginald A. Valdez¹, Michael B. Stadler ^{2,5}, Dirk G. de Rooij⁸, Paola Capodiec  ³, John Tallarico ¹, Antoine H. F. M. Peters ^{2,4,10} & Thomas B. Nicholson ^{3,10} **

¹Discovery Sciences, Novartis Biomedical Research, Cambridge, MA, USA. ²Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland. ³Disease Area X (DAX), Novartis Biomedical Research, Cambridge, MA, USA. ⁴Faculty of Sciences, University of Basel, Basel, Switzerland. ⁵Swiss Institute of Bioinformatics, Basel, Switzerland. ⁶Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan. ⁷Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, Texas, USA. ⁸Reproductive Biology Group, Division of Developmental Biology, Department of Biology, Faculty of Science, Utrecht University, Utrecht, the Netherlands. ⁹These authors contributed equally: Michael T. Bocker, Grigorios Fanourgakis. ¹⁰These authors jointly supervised this work: Antoine H. F. M. Peters, Thomas B. Nicholson. ✉ e-mail: antoine.peters@fmi.ch; thomas.nicholson@novartis.com

Gene regulation by Polycomb Repressive Complexes



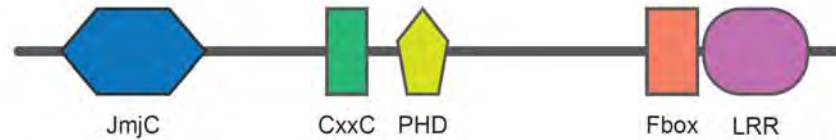
Repressive histone modifications, **H2AK119ub (PRC1)** and **H3K27me3 (PRC2)**.

Gene repression in male germ cells, oocytes and embryos, imprinted X inactivation and placenta development.

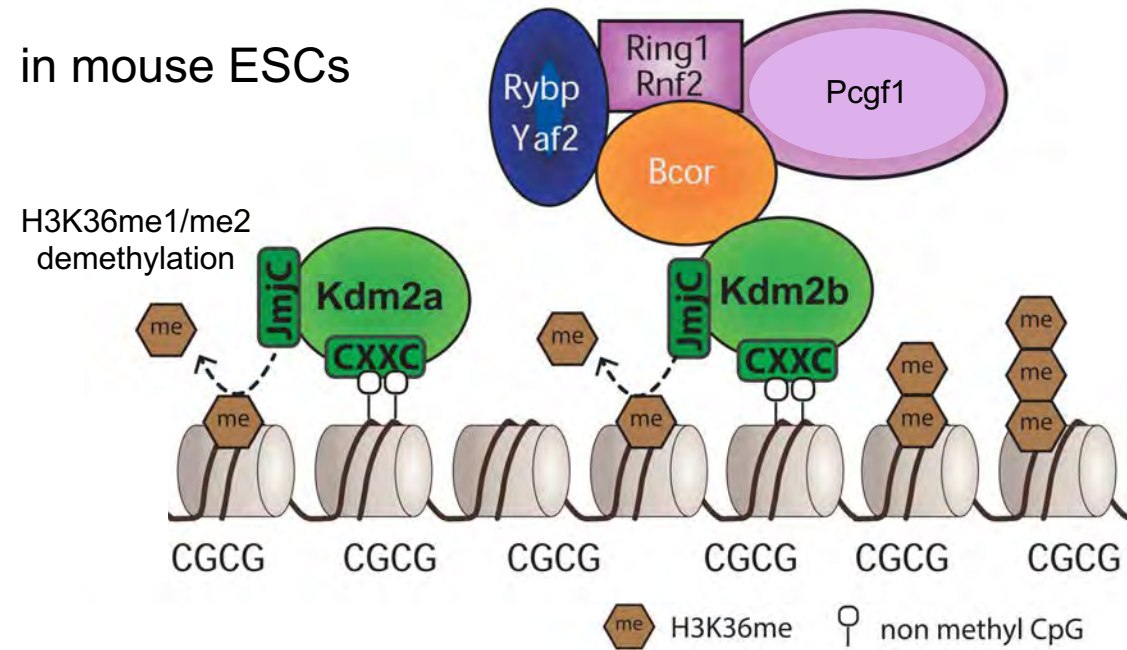
3D Polycomb Associated Domain (PAD) formation in oocytes and embryos

KDM2B prevents DNAm at PRC1/PRC2-controlled CGI promoters in mESCs

Protein domain structure



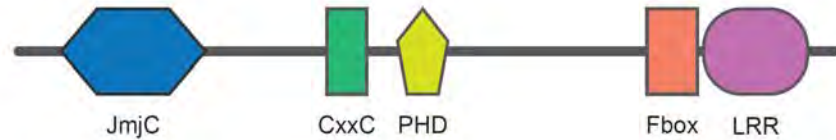
in mouse ESCs



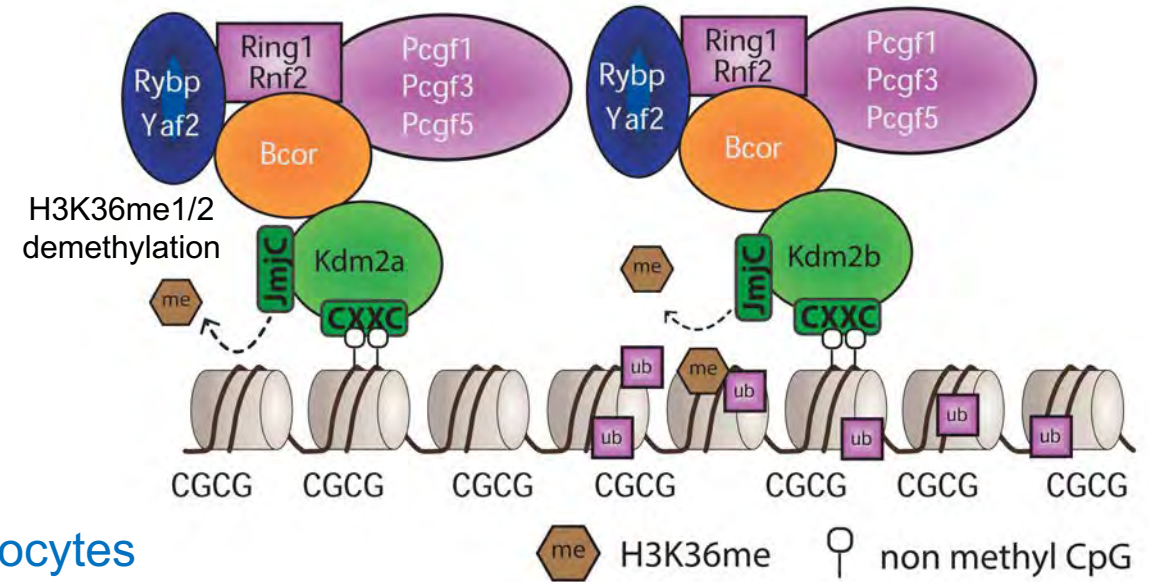
- KDM2A and KDM2B demethylate H3K36me1/me2 through the **JmjC** domain.
- In ESCs, KDM2B interacts with vPRC1.1 proteins, while KDM2A does not.
- KDM2A and KDM2B have a **CxxC** domain, which binds to all non-methylated CpG islands, irrespective of whether being transcriptionally active or repressed by PRC1/2 repression.
- In ESCs, *Kdm2b* prevents DNA methylation at PRC1 regulated CGI-promoter genes.

Conditional deficiency study of *Kdm2a* and *Kdm2b* function in oocytes

protein domain structure

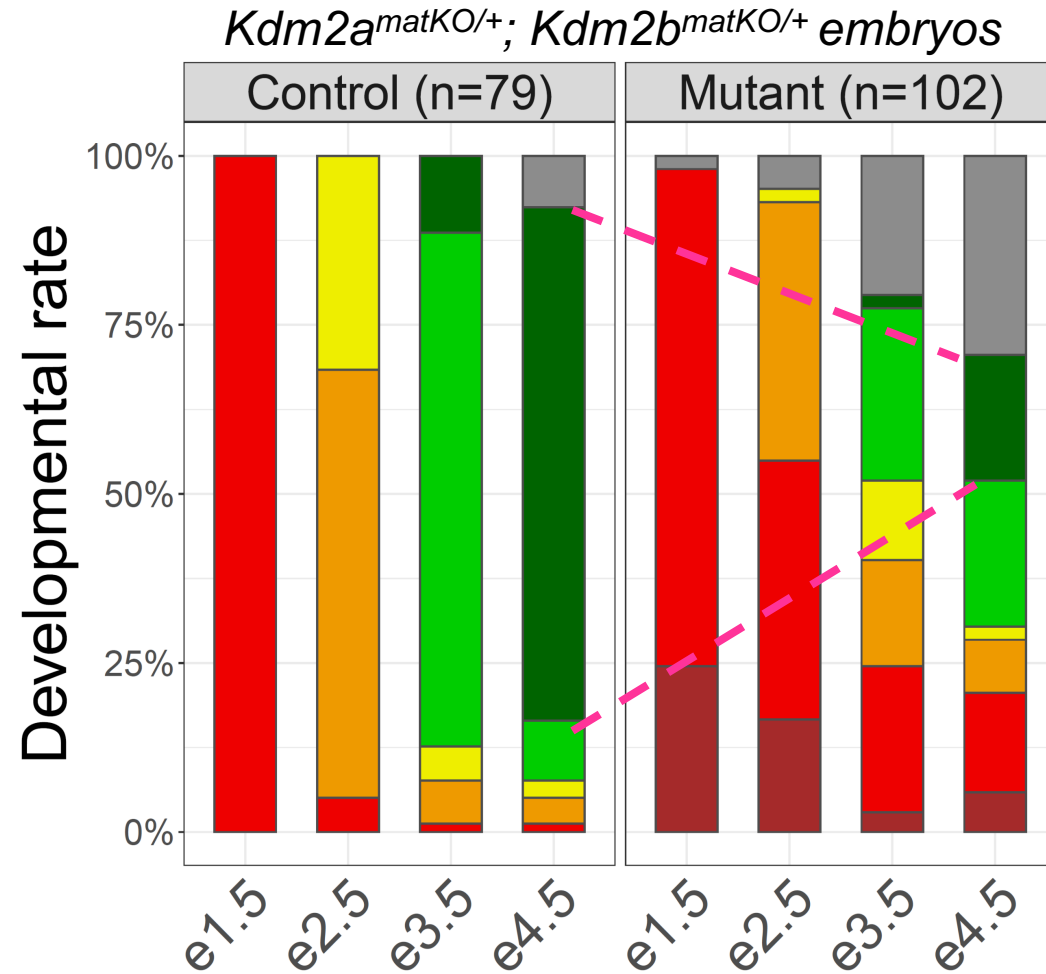


in mouse oocytes

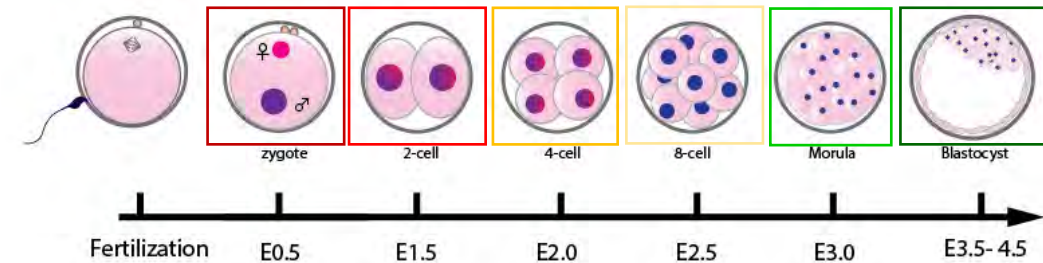


- *Kdm2a* and *Kdm2b* are both expressed in growing oocytes
- Mutations: *Kdm2a* floxed JmjC → KO / null allele
Kdm2b floxed JmjC → KO / null allele
Kdm2b floxed CxxC → in frame deletion; no recruitment to unmethylated CpGs

Kdm2a/Kdm2b double deficiency in oocytes impairs embryonic development



mutant oocyte
X
wt sperm



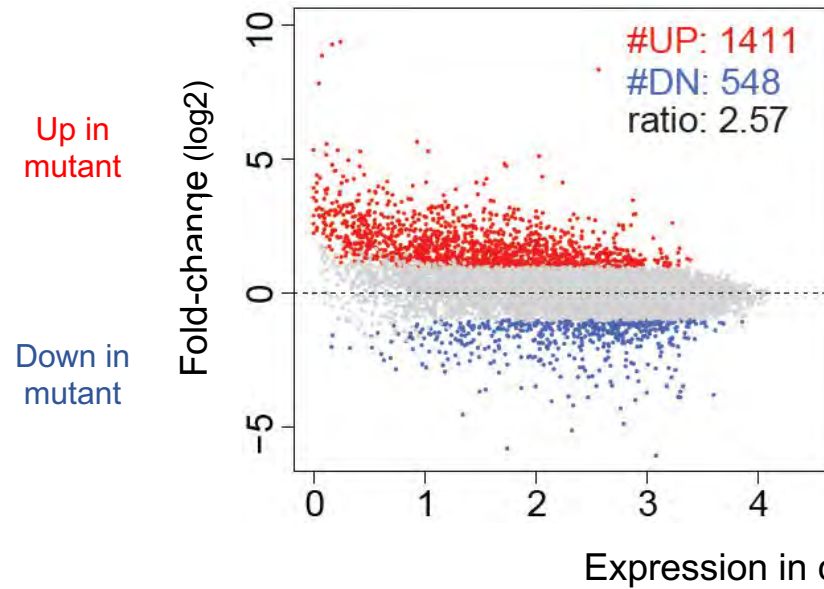
stage

zygote	4/6-cell	morula	fragmented
2/3-cell	8-cell	blastocyst	

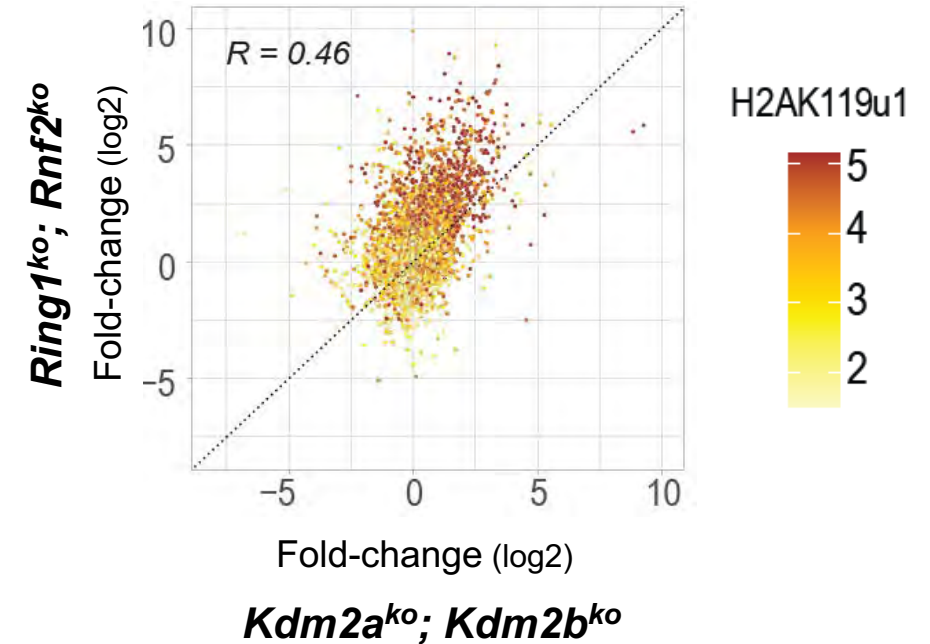
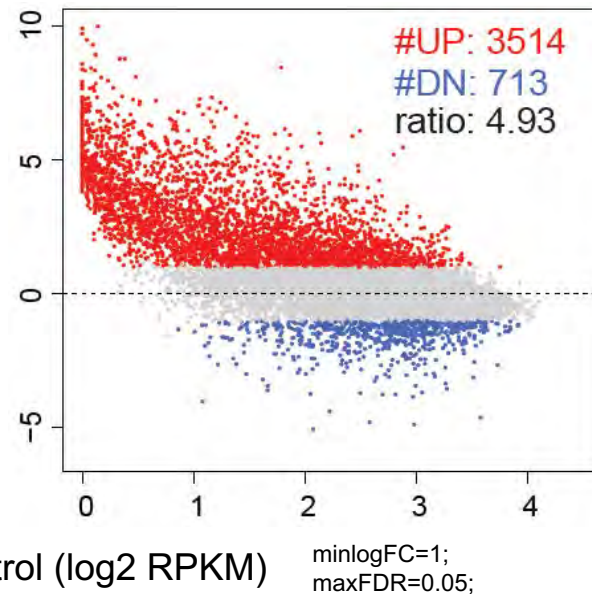
KDM2A/KDM2B proteins repress PRC1 target genes in oocytes

(KO for all PRC1 complexes)

Kdm2a^{ko}; Kdm2b^{ko}

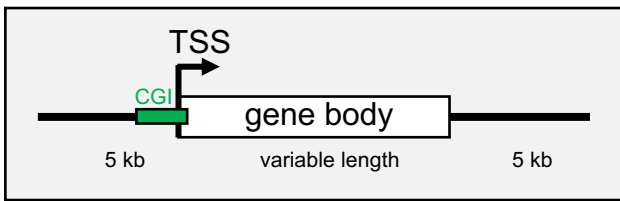


Ring1^{ko}; Rnf2^{ko}

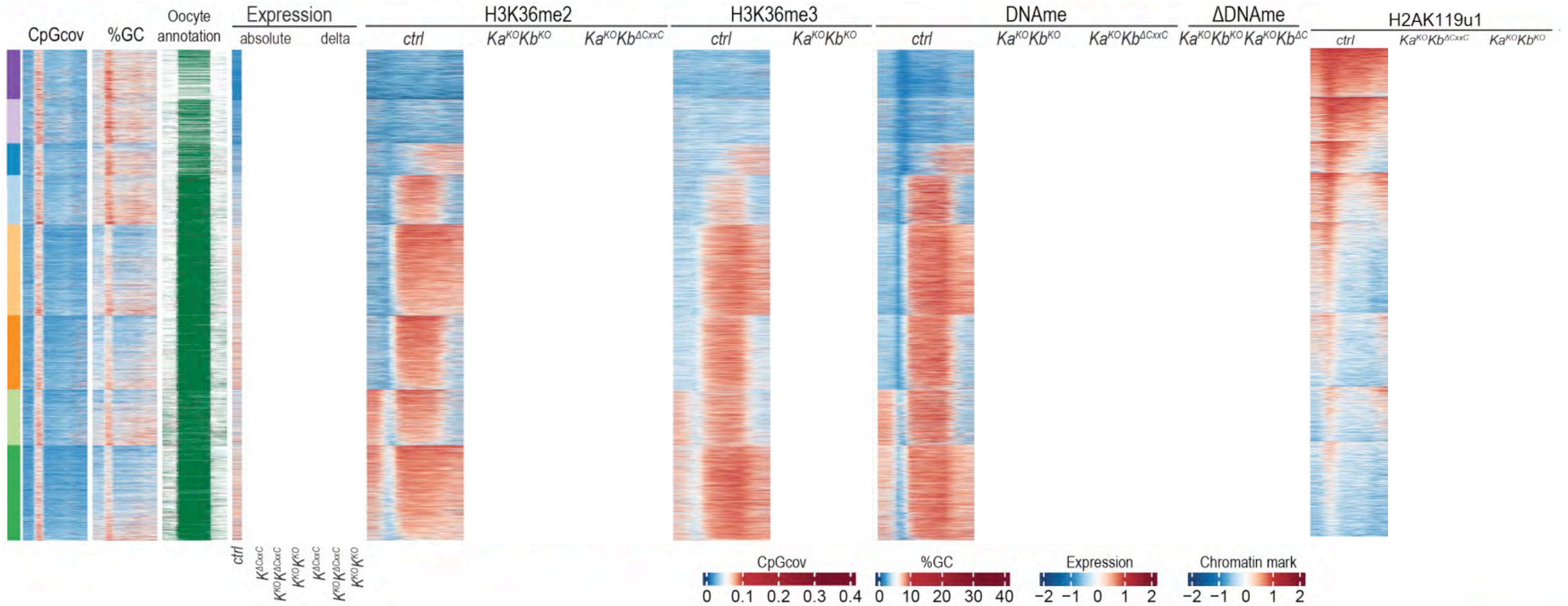


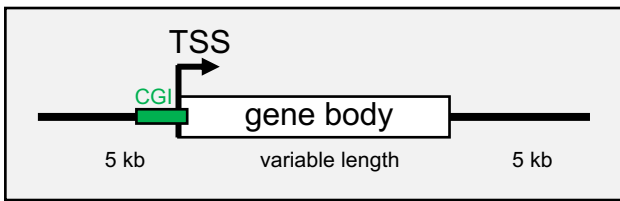
Smart-seq2 RNA sequencing : sequencing of ~20 oocytes per genotype

- Comparable but less extensive defects in gene repression in *Kdm2a/Kdm2b* dko mutant oocytes versus in *Ring1/Rnf2* dko oocytes.
- Up-regulated genes are enriched for H2Au1.
- Upregulated genes include “developmental genes” and other typical “Polycomb target genes”.

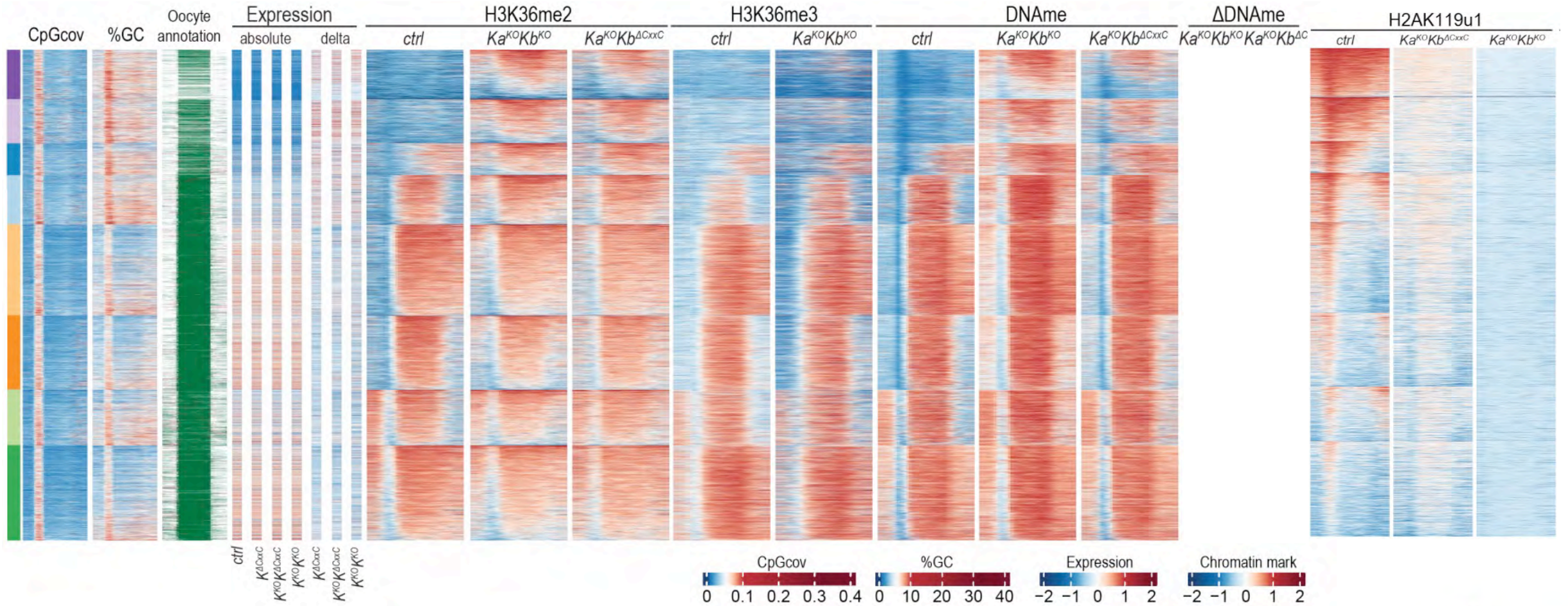


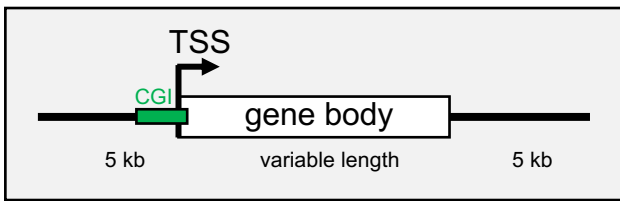
H3K36me2, H3K36me3 and DNA methylation are deposited along expressed genes in control oocytes



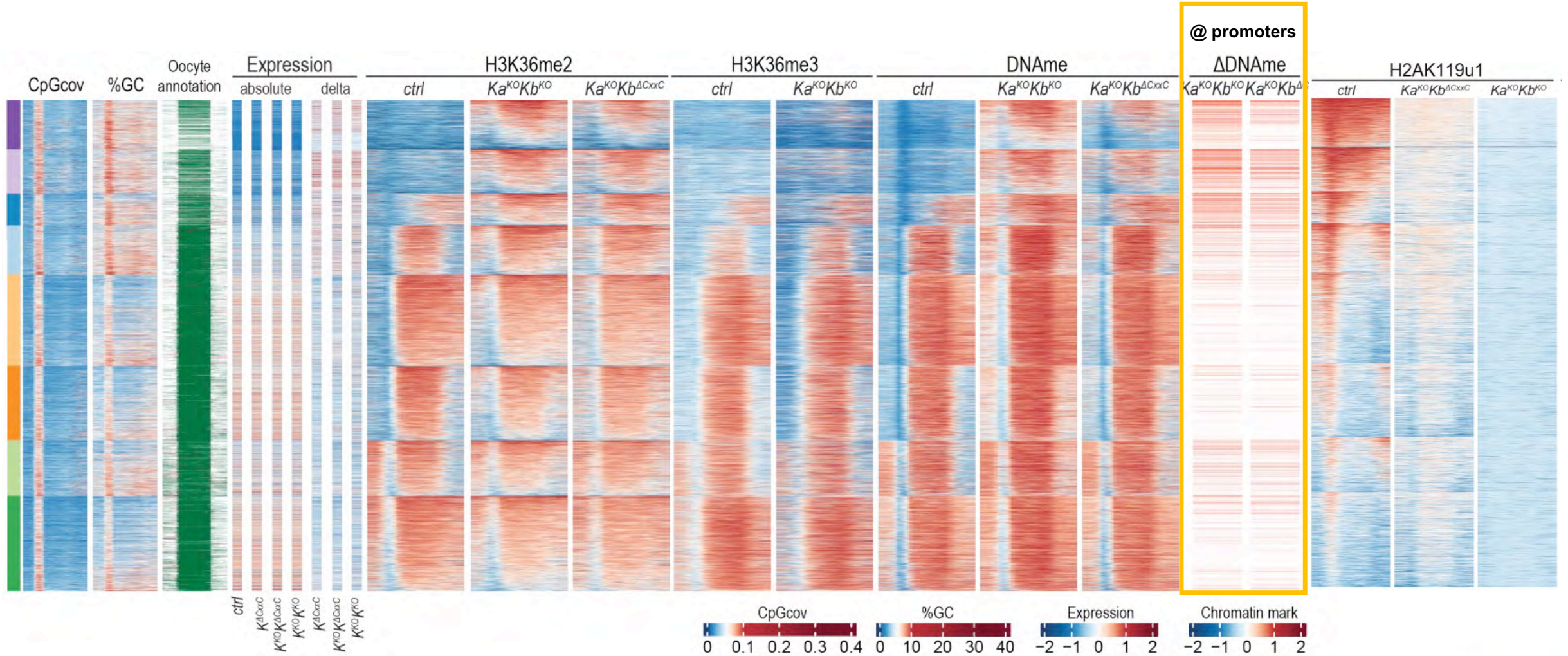


Accumulation of H3K36me2 and DNA methylation at Polycomb-regulated genes in mutant oocytes

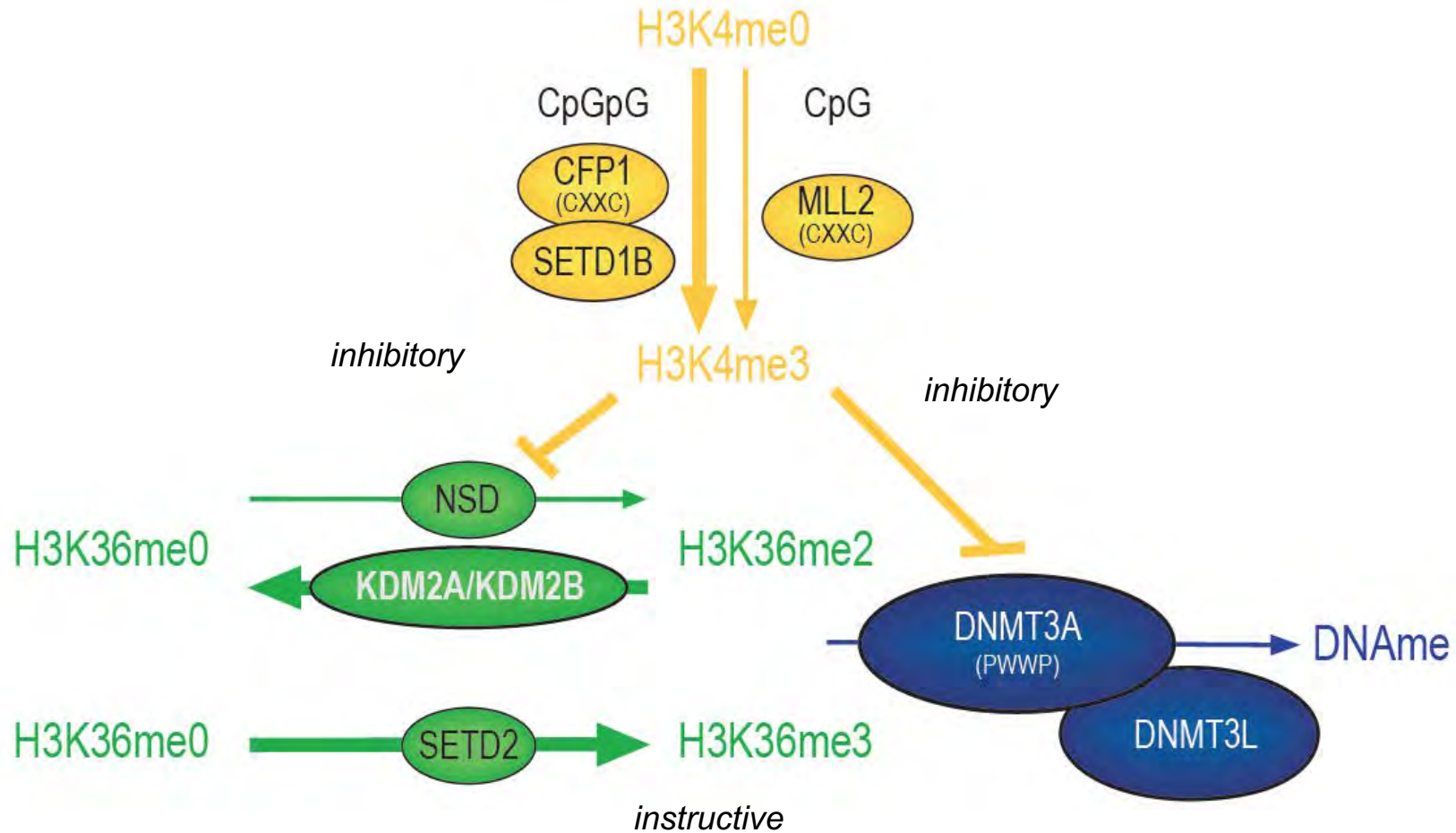




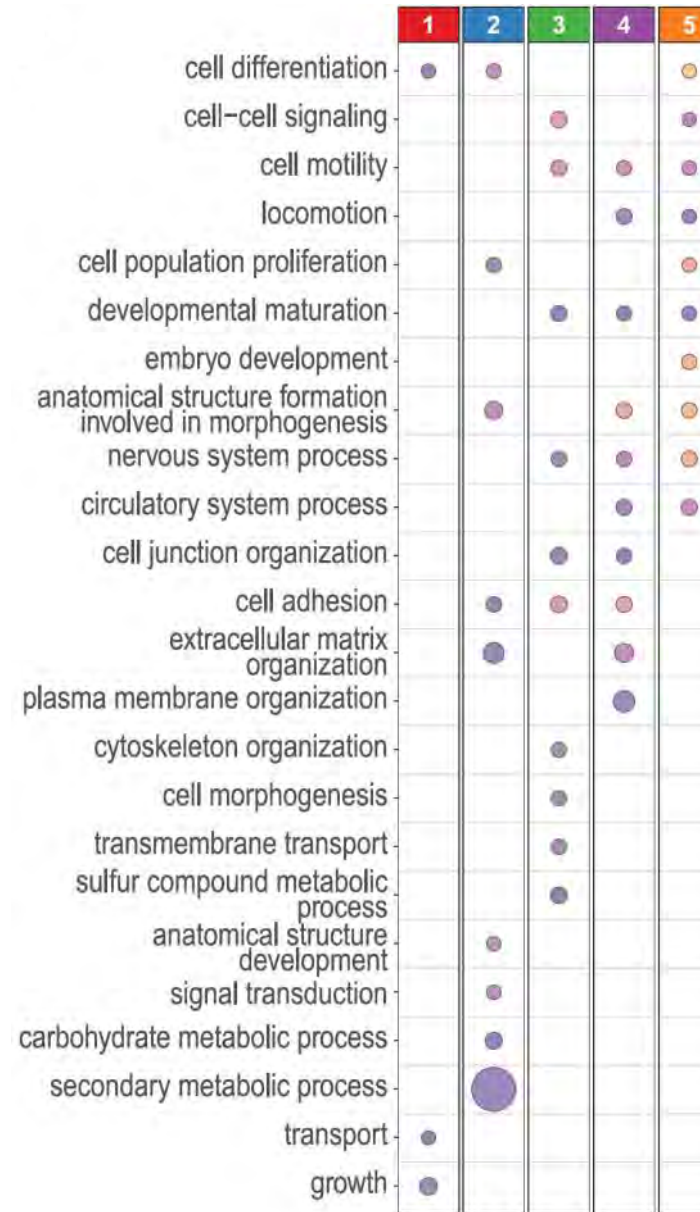
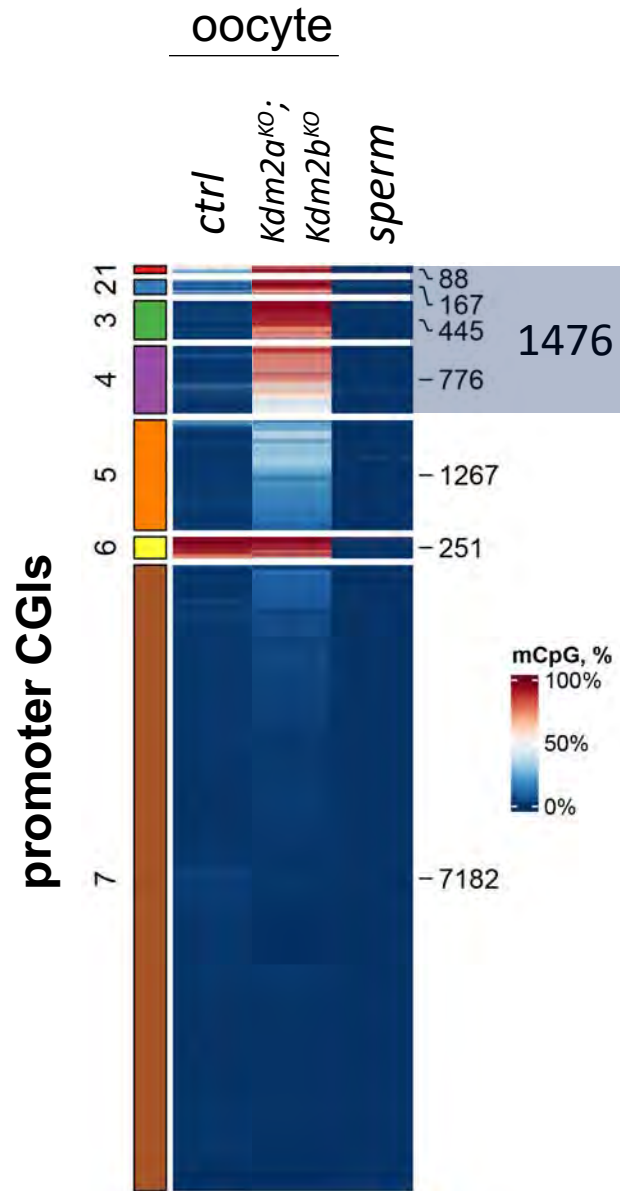
Accumulation of DNA methylation at CGI promoters of PRC1 target and non-target genes in mutant oocytes



Sequence and chromatin features define H3K36me2 occupancy and DNAm at CGIs



Aberrant DNA methylation is present at promoter sequences of developmental regulatory genes



Hyper DNA methylation at promoters of genes driving development

Gene Ontology analysis:

cell fate specification and determination, morphogenesis, cellular differentiation and cell cycle regulation

Gene families:

transcription factors: *Foxo*, *Gata*, *Hand*, *Hes*, *Hox*, *Lhx*, *Nkx*, *Pax*, *Six*, *Sox*, *Tead*, *Tbx*, and *Zfp*.

cell signaling, growth factor activity, and cell adhesion: *Bmp*, *Dll*, *Fgf*, *Igf2*, *Pdgf*, *Pcdh*, *Rar*, *Wnt*, and *Tgfb*),

gonadal development and synaptonemal complex assembly

Developmental processes:

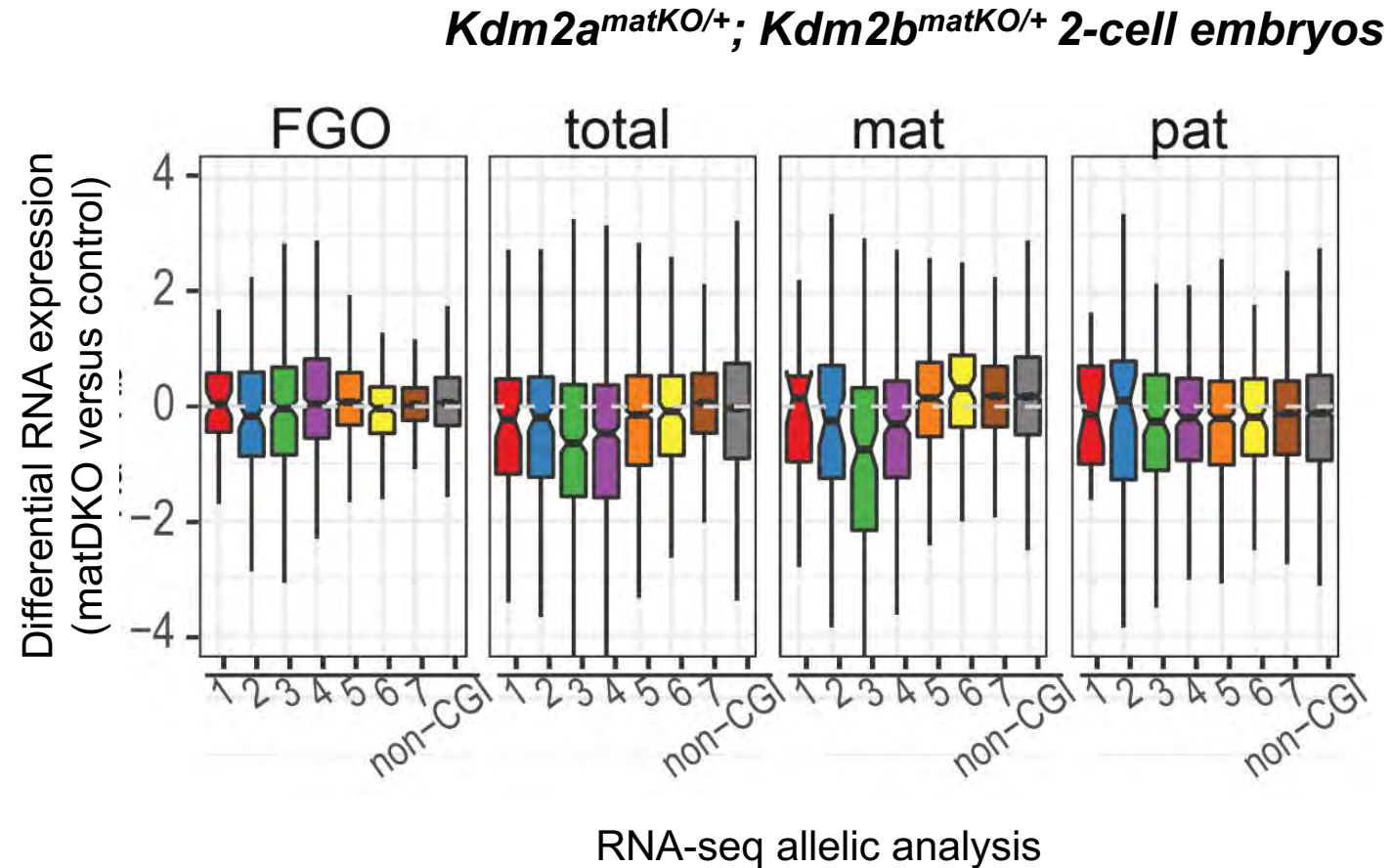
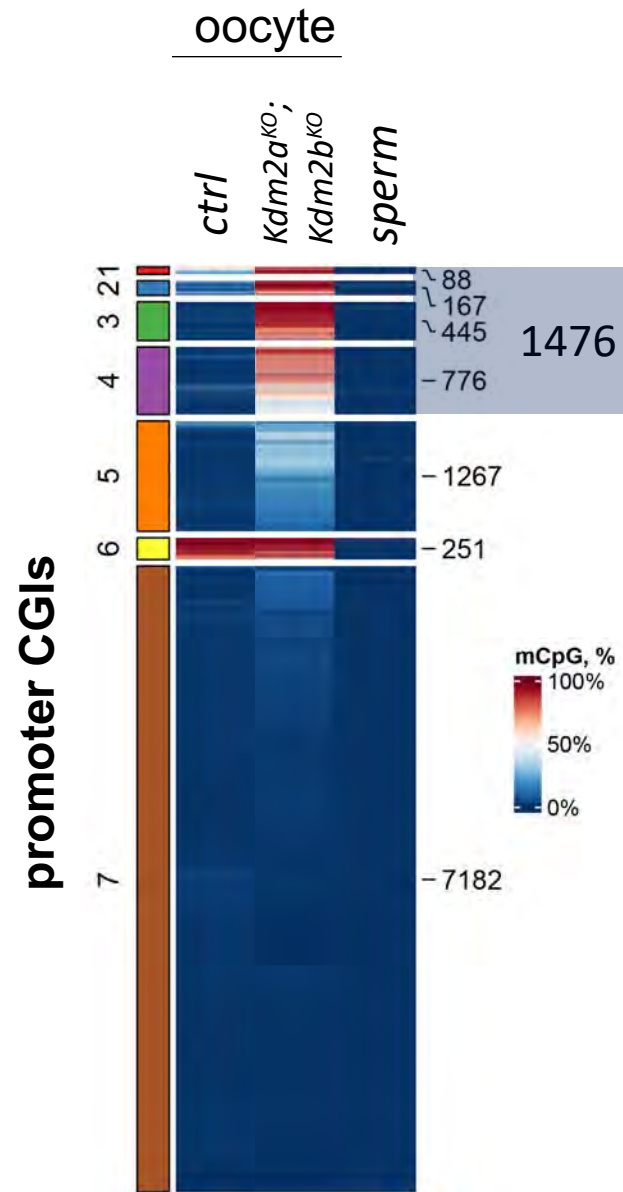
blastocyst development: *Eomes*, *Gata4*, *Hnf1b*, *Junb*, *Klf4*, and *Sox17*

placenta development: *Atrx*, *Hand1*, *Lhx3*, *Vash2*, and *Wnt2*

Gastrulation: *Bmp4*, *Brachyury* [*T*], *Sox7*, and *Tlx2*

similar to genes
lowly expressed
in gonads of
HIR patients

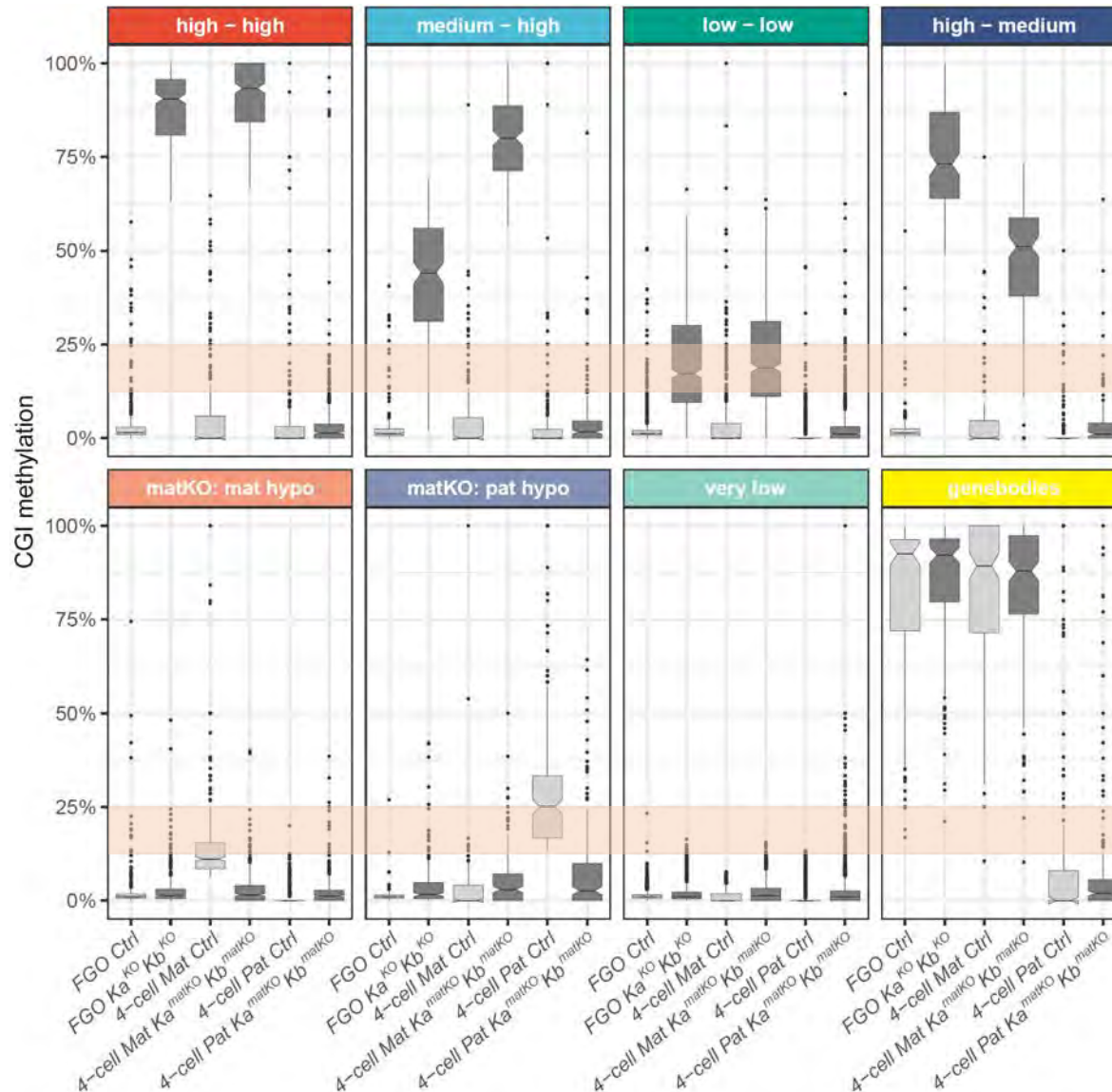
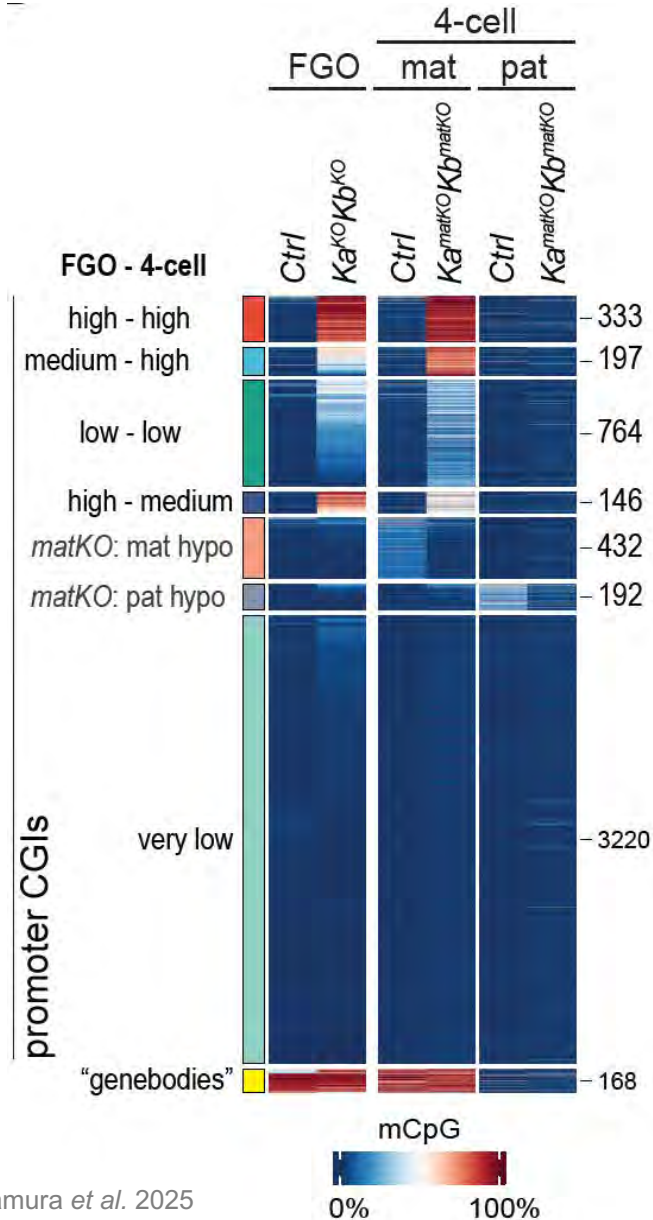
Aberrant DNA methylation is associated with transcriptional down-regulation of maternal alleles in 2-cell embryos



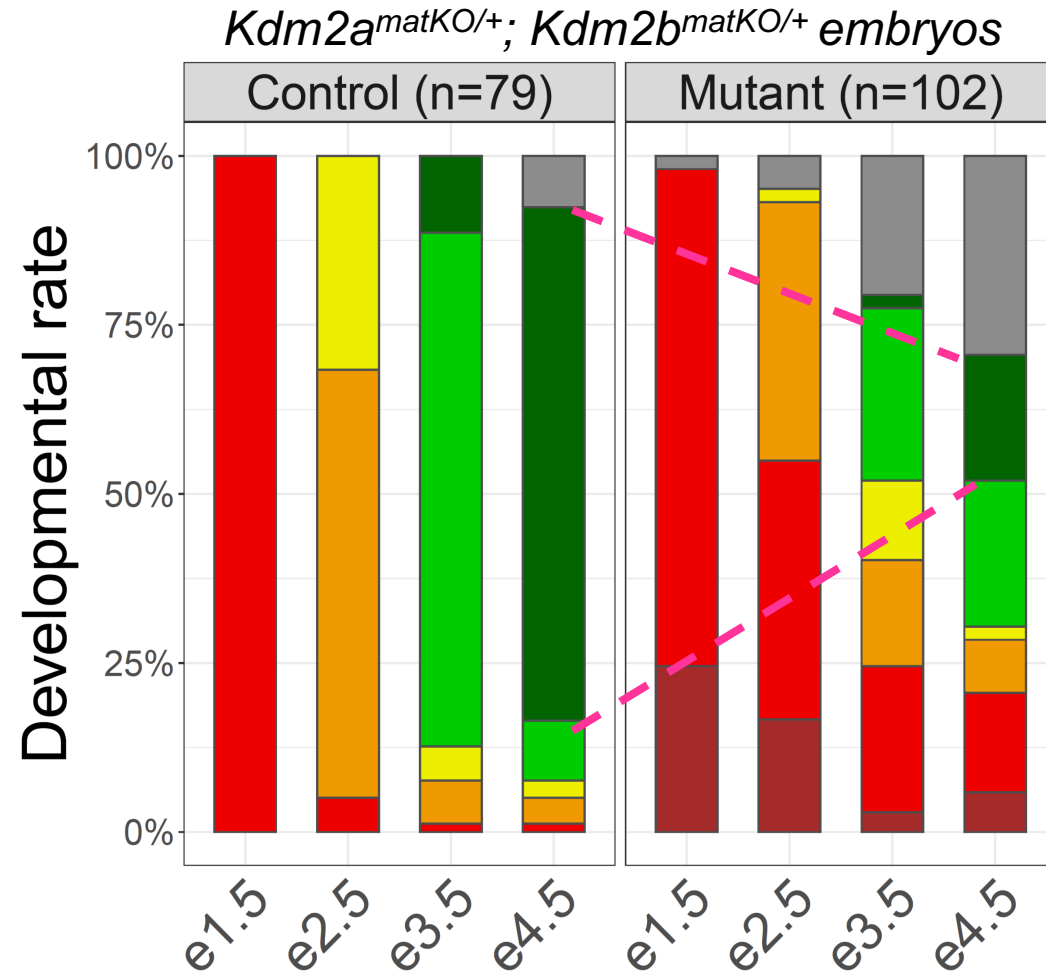
Maternal DNA methylation @ promoter transmission

Gene expression from maternal allele @ 2-cell

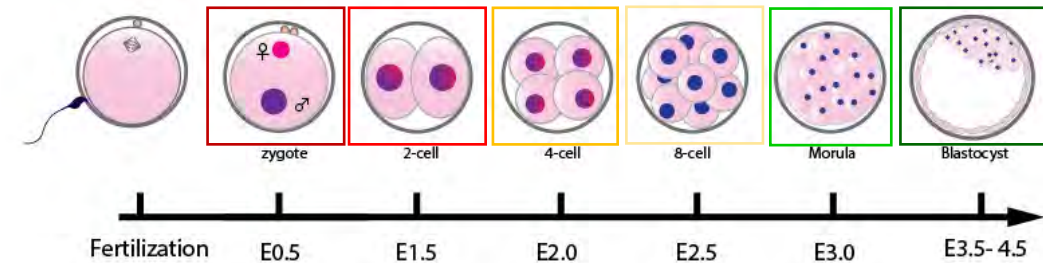
Aberrant maternal DNA methylation is maintained, even enhanced or somewhat reduced in 4-cell embryos



Kdm2a/Kdm2b double deficiency in oocytes impairs embryonic development



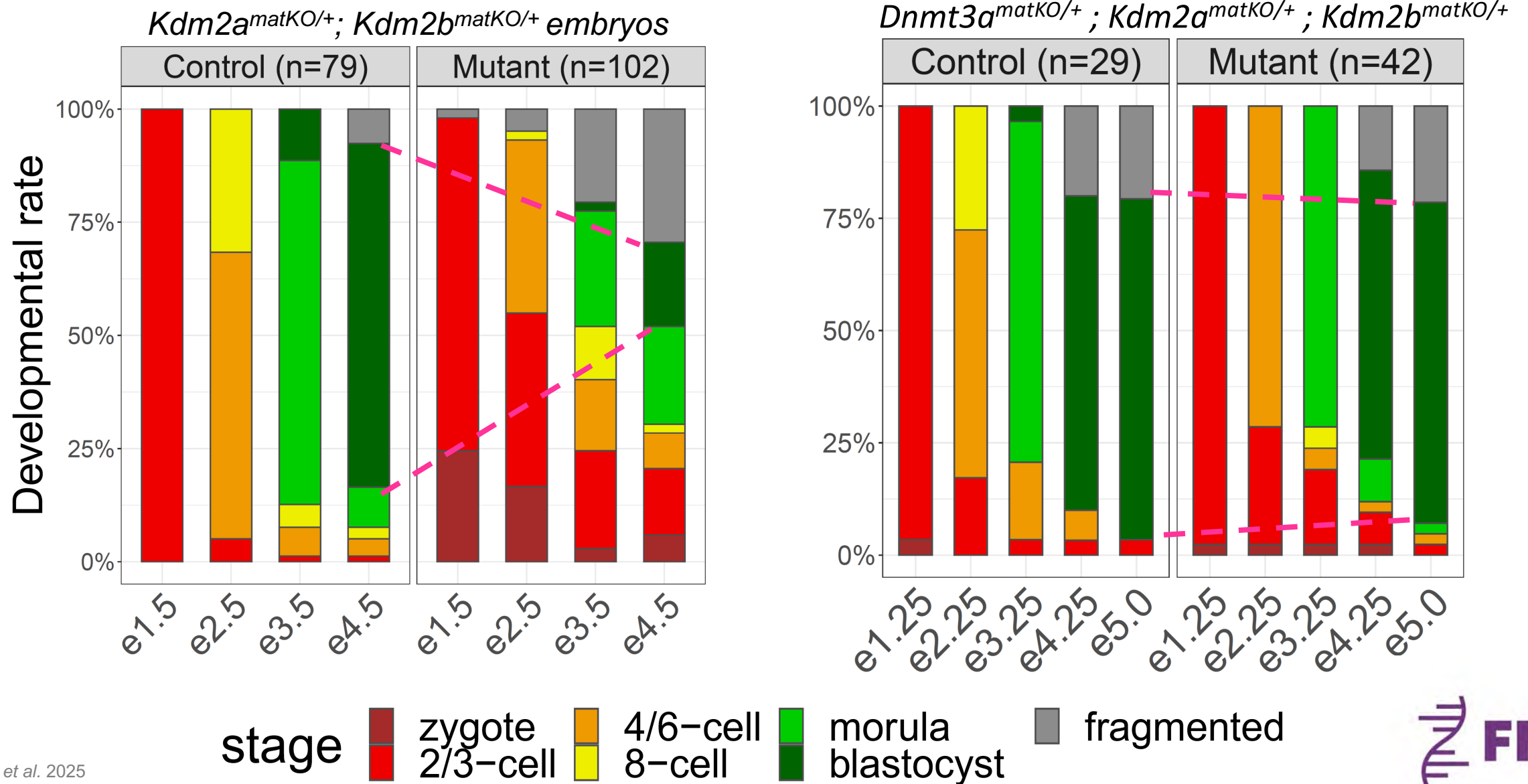
mutant oocyte
X
wt sperm



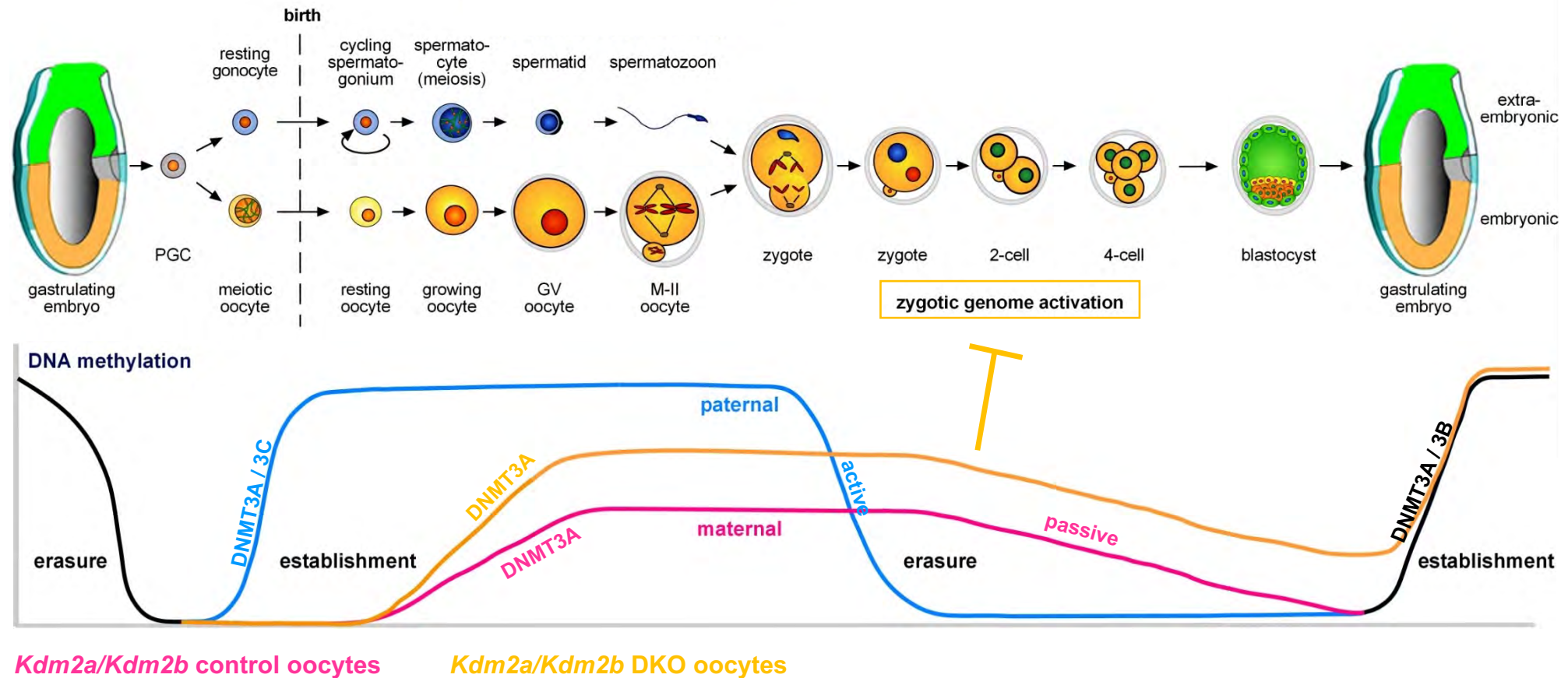
stage

zygote	4/6-cell	morula	fragmented
2/3-cell	8-cell	blastocyst	

Dnmt3a (but not *Dnmt1*) deficiency suppresses lethality of *Kdm2a/Kdm2b* maternally deficient pre-implantation embryos



KDM2A/KDM2B proteins restrict DNMT3A *de novo* methylation in oocytes to support pre-implantation development



KDM2A/KDM2B function in oocytes as developmental gatekeepers, preventing intergenerational inheritance of faulty DNA methylation.

KDM2A/KDM2B are key members of a chromatin modifier network possibly conserving CpG dense-regions throughout evolution.

Take home messages: germ line DNA methylation, early development and CpG island conservation

Observations:

Too much CpG methylation in the maternal genome, established during oogenesis, is detrimental for pre-implantation development.

Aberrant *de novo* DNA methylation occurs at > 1500 CGI promoters as well as throughout the genome.

Aberrant methylation at CGI promoters measured in oocytes and 4-cell embryos is associated with maternal allele specific gene repression in 2-cell embryos, indicating an inhibitory role in zygotic gene regulation.

Implications:

DNA demethylation activities present in early embryos are not sufficient for resetting aberrant maternal DNA methylation (TET3, other mechanisms). Contrary, we observe efficient maintenance of aberrant CGI methylation in embryos (in part also seen in wildtype embryos as well - Smallwood et al., 2011).

Early embryonic lethality argues for haplo-insufficiency of certain gene functions during pre-implantation development.

One open question is whether the identified histone methylation network contributed to the formation and retention of CpG islands in mammalian genomes?

Chromatin network interactions controlling DNA methylation levels at CpG-rich sequences, including CpG islands at promoters and in gene bodies

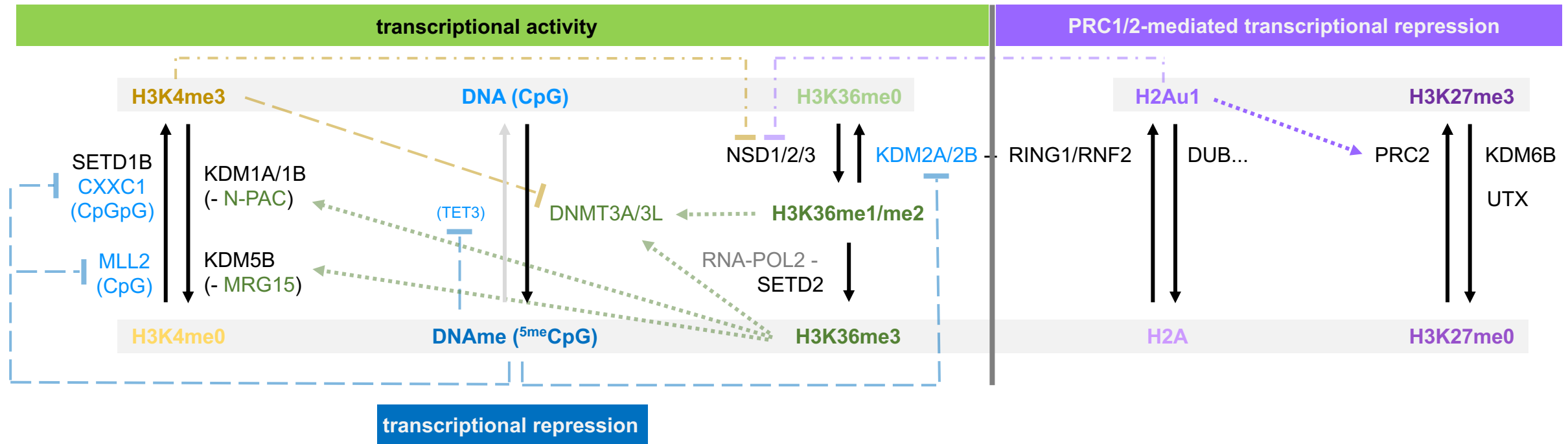


Table 2. New identified genes downregulated in HIR and involved in the HPG axis

Gene ID	Name	HIR median	LIR median	logFC ^a	FDR
ARID3B	AT rich interactive domain 3B (BRIGHT-like)	1.35	3.35	-1.44965	0.000171
ATF3	activating transcription factor 3			-0.96099	0.009544
AXIN2	axin 2			-1.28981	0.000283
BMP7	bone morphogenetic protein 7			-1.58963	0.00144
BMPR1B	bone morphogenetic protein receptor, type IB			-2.5653	0.000184
CHD7	chromodomain helicase DNA binding protein 7			-0.72742	0.001522
CLOCK	clock circadian regulator			-0.46419	0.01024
DLX2	distal-less homeobox 2			-2.60142	0.003894
DLX3	distal-less homeobox 3			-2.07726	0.006612
DLX5	distal-less homeobox 5			-1.92109	0.00407
DUSP4	dual specificity phosphatase 4			-1.73444	0.000154
EBF3	early B-cell factor 3			-1.94656	0.000218
EGR2	early growth response 2			-1.17858	0.001333
FGF14	fibroblast growth factor 14			-1.23942	0.020814
FGFR1	fibroblast growth factor receptor 1			-0.49372	0.024252
FGFR2	fibroblast growth factor receptor 2			-1.25292	0.00052
FGFRL1	fibroblast growth factor receptor-like 1			-0.97919	0.010591
GATA2	GATA binding protein 2			-1.52714	0.000339
ISL2	ISL LIM homeobox 2			-1.6183	0.008855
KLF4	Kruppel-like factor 4 (gut)	1.43	4.11	-1.16046	0.008282
LHX2	LIM homeobox 2	0.03	0.28	-3.13022	0.0019
LHX6	LIM homeobox 6	0.35	1.17	-1.72084	0.000238

Many genes are the typical developmental regulatory genes including transcription factors, signalling molecules as well as typical germ line expressed genes

Cryptorchidism:
Failed
upregulation of
novel genes
involved in the
HPG axis in
gonadal samples
of high infertility
risk patients.

Absence of HPG-mediated activation of genes in HIR gonocytes?

Intrinsically impaired activation and/or aberrant repression during differentiation towards spermatogonial fate?

Future experiments on HIR and LIR patient samples:

- Single cell RNA-seq
- Germ cell sorting?
- H2AK119u1 and H3K27me3 profiling
- DNA methylation profiling

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Maarten van Lohuizen

Stuart Orkin

En Li

FMI facilities

Functional genomics

Computational biology

FAIM

Transgenic facility

Animal facility

FACS facility

Proteomics



Friedrich Miescher Institute
for Biomedical Research

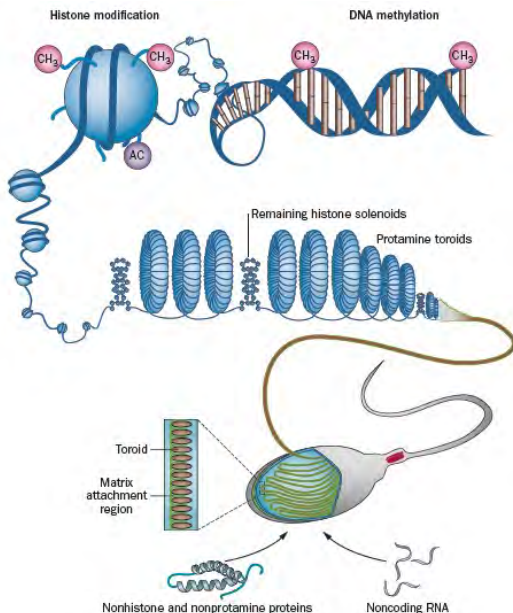
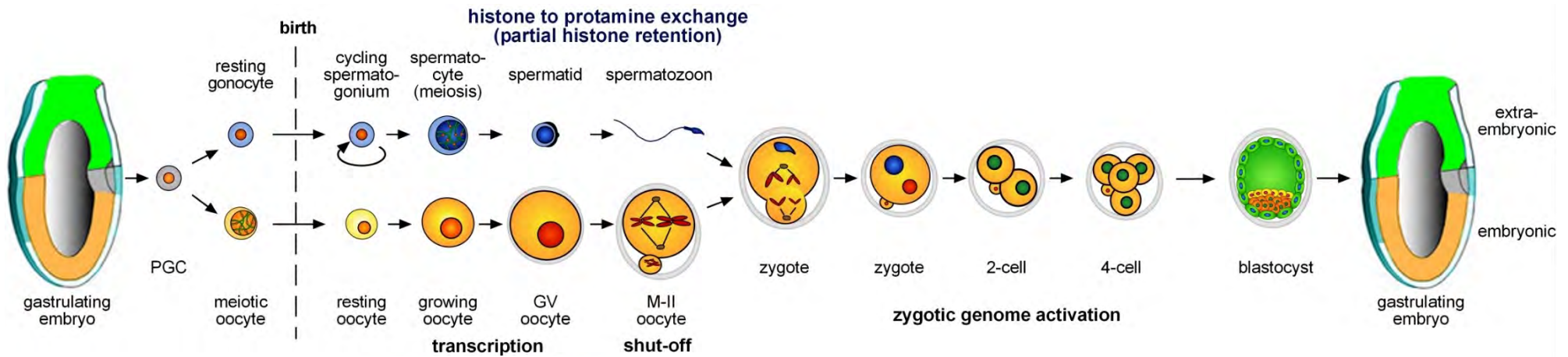


JSPS Research Fellow for Abroad
Naito Foundation



European Research Council
Established by the European Commission

Extensive chromatin remodeling in the male germ line



Last decade: ChIP-seq studies showed that nucleosomes are present in mammalian sperm – yet genomic location has been majorly debated (Cairns, Peters, Corces, Okada and Rando labs).

Our recent findings:

- Single molecule chromatin footprinting analysis show that nucleosomes are present in sperm chromatin at low frequencies (Gaspa-Toneu, Shi *et al.*).
- DNA methylation in developing male germ cells promotes loss of nucleosomes during spermatid elongation (Erkek *et al.*, 2013; Fanourgakis *et al.*, 2025).
- DNA methylation inherited from sperm prevents early deposition of H3K4me3 at regulatory sequences in early mouse embryos (Fanourgakis *et al.*, 2025).

Table 1. Genes involved in germ cell development and in the HPG axis (bold) and downregulated in the HIR group

Gene ID	Name	logFC ^a	FDR	pHAZR/LAZR
ALDH1A2	aldehyde dehydrogenase 1 family, member A2	-2.36946	0.000175	0.0006
AMPH	amphiphysin	-1.51873	0.000888	0.02
CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	-1.87634	0.00024	0.0007
CBL	Cbl proto-oncogene, E3 ubiquitin protein ligase	-1.28185	0.0007	0.0004
CDC20	cell division cycle 20	-2.5884	0.000154	0.003
CLGN	calmegin	-1.04018	0.014042	0.01
CSRP2	cysteine and glycine-rich protein 2	-1.79674	0.000264	0.002
CXCL9	chemokine (C-X-C motif) ligand 9	-1.98042	0.00121	0.0007
DAZ1	deleted in azoospermia 1	-2.08964	0.004096	n.s.
DAZL	deleted in azoospermia-like	-1.30306	0.007261	0.03
DDX25	DEAD (Asp-Glu-Ala-Asp) box helicase 25	-0.97862	0.003528	0.001
DDX4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4	-2.86159	0.000222	8.25E-05
DMRTB1	DMRT-like family B with proline-rich C-terminal, 1	-3.26495	0.000154	0.003
DPYSL4	dihydropyrimidinase-like 4	-1.09804	0.02908	0.001
DTL	denticless E3 ubiquitin protein ligase homolog (<i>Drosophila</i>)	-1.51525	0.00036	0.0007
DUSP5	dual specificity phosphatase 5	-2.14862	0.000267	0.008
EGR4	early growth response 4	-3.35225	0.000322	0.006
ESX1	ESX homeobox 1	-3.34104	0.000154	8.63E-05
FGF9	fibroblast growth factor 9	-1.06052	0.001631	0.0001
FGFR3	fibroblast growth factor receptor 3	-3.32791	0.000154	0.0001
FOXP1	forkhead box G1	-1.60584	0.004397	1.21E-05
FST	follicle-stimulating hormone receptor	-1.99538	0.000624	0.001
GAGE1	G antigen 1	-1.79238	0.007285	0.001
GTSE1	gametocyte-specific factor 1	-2.96989	0.000154	0.0001
ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	-1.53417	0.001103	0.0002
ISL1	ISL LIM homeobox 1	-2.09731	0.000515	0.0004
KIF21B	kinesin family member 21B	-1.9747	0.000218	0.0001
LIN28B	lin-28 homolog B (<i>C. elegans</i>)	-2.2908	0.000264	0.01
LIN7B	lin-7 homolog B (<i>C. elegans</i>)	-1.94848	0.000322	0.02
MAGEA4	melanoma antigen family A4	-2.65916	0.000218	0.009
MAGEC1	melanoma antigen family C1	-2.50237	0.000154	8.24E-05
MND1	meiotic nuclear divisions 1 homolog (<i>S. cerevisiae</i>)	-2.18184	0.000547	0.004
MORC1	MORC family CW-type zinc finger 1	-2.46752	0.000281	0.0001
NLRP2	NLR family, pyrin domain containing 2	-1.4955	0.001143	0.005
NMU	neuromedin U	-2.1983	0.001858	0.005
NRG1	neuregulin 1	-0.92134	0.013631	0.004
PIWIL2	piwi-like RNA-mediated gene silencing 2	-1.75871	0.000877	0.0001
PIWIL4	piwi-like RNA-mediated gene silencing 4	-2.08022	0.000154	0.0001
RGS7	regulator of G-protein signaling 7	-1.6375	0.001783	0.001
RNF17	ring finger protein 17	-2.36921	0.001931	0.01
SH3GL2	SH3-domain GRB2-like 2	-1.42897	0.000322	0.008
SIX1	SIX homeobox 1	-2.53474	0.000218	0.02
SNAP91	synaptosomal-associated protein, 91 kDa	-1.71458	0.00038	0.008
SOX30	SRY (sex-determining region Y)-box 30	-1.94278	0.00025	0.002
SPA17	sperm autoantigenic protein 17	-1.85214	0.001258	0.01
SSX2	synovial sarcoma, X breakpoint 2	-2.02297	0.001142	0.003
SYCP3	synaptonemal complex protein 3	-2.24491	0.002046	0.0002
TAF5	TAF5 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 100 kDa	-1.08538	0.000894	0.005
TAF7L	TAF7-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 50 kDa	-1.98595	0.001673	0.03
TDRD10	tudor domain containing 10	-1.80786	0.000981	n.s.
TDRD5	tudor domain containing 5	-2.64116	0.000294	0.01
TDRD6	tudor domain containing 6	-1.80614	0.000154	0.0003
TDRD9	tudor domain containing 9	-1.52239	0.00057	0.009
TEX14	testis expressed 14	-1.53753	0.000453	0.001
TLE1	transducin-like enhancer of split 1 (E(sp1) homolog, <i>Drosophila</i>)	-0.90022	0.001061	0.0001
TSPY1	testis-specific protein, Y-linked 1	-2.49393	0.000323	8.24E-05
WNT3	wingless-type MMTV integration site family, member 3	-2.64239	0.000154	0.0003
MBD2	methyl-CpG binding domain protein 2	n.s.	n.s.	0.03
SRY	sex-determining region Y	n.s.	n.s.	0.002
UTF1	undifferentiated embryonic cell transcription factor 1	n.s.	n.s.	0.0002
RBMY1A1	RNA binding motif protein, Y-linked, family 1, member A1	NA	NA	0.0008
RBMY1B	RNA binding motif protein, Y-linked, family 1, member B	-1.93256	0.000405	
RBMY1E	RNA binding motif protein, Y-linked, family 1, member E	-1.90322	0.002026	
RBMY1F	RNA binding motif protein, Y-linked, family 1, member F	-2.02817	0.000822	
RBMY1J	RNA binding motif protein, Y-linked, family 1, member J	-1.95224	0.000653	

Table adapted from Hadziselimovic et al. [2009].

^a Absolute fold-changes <2 are highlighted in italics.

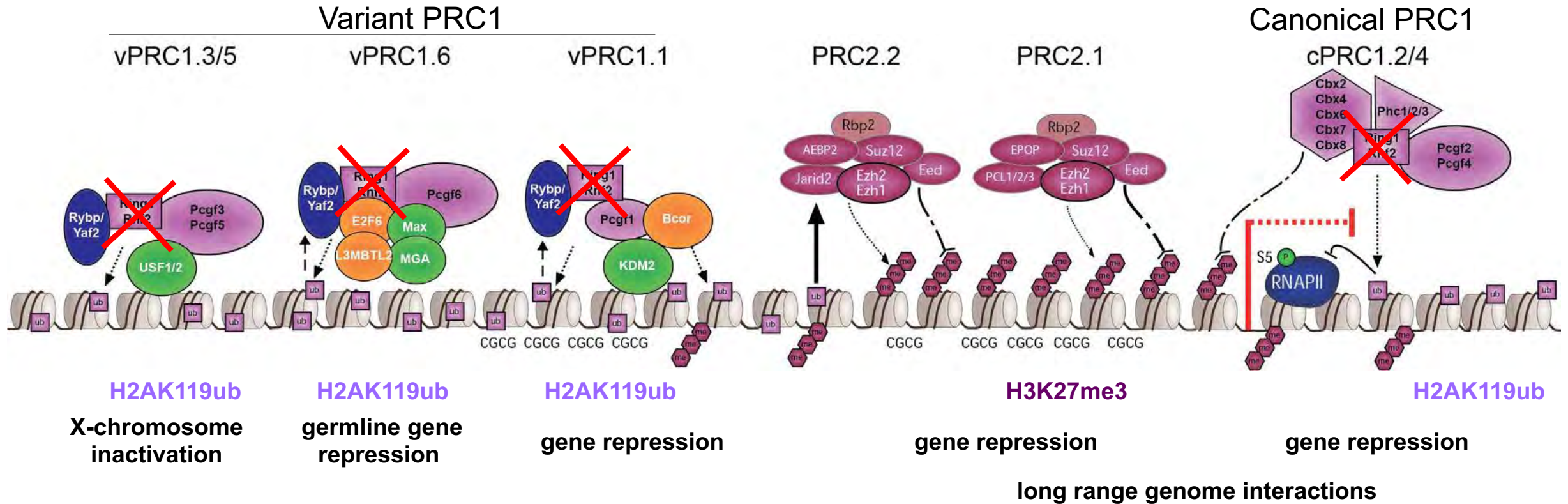
Downregulation of genes known to be involved in germ cell development and in the HPG axis in gonadal samples of high infertility risk patients.

Many genes are the typical developmental regulatory genes including transcription factors, signalling molecules as well as typical germline expressed genes.

Maternal deficiency for *Ring1* and *Rnf2* results in an arrest at the 2-cell stage

initiation of silencing

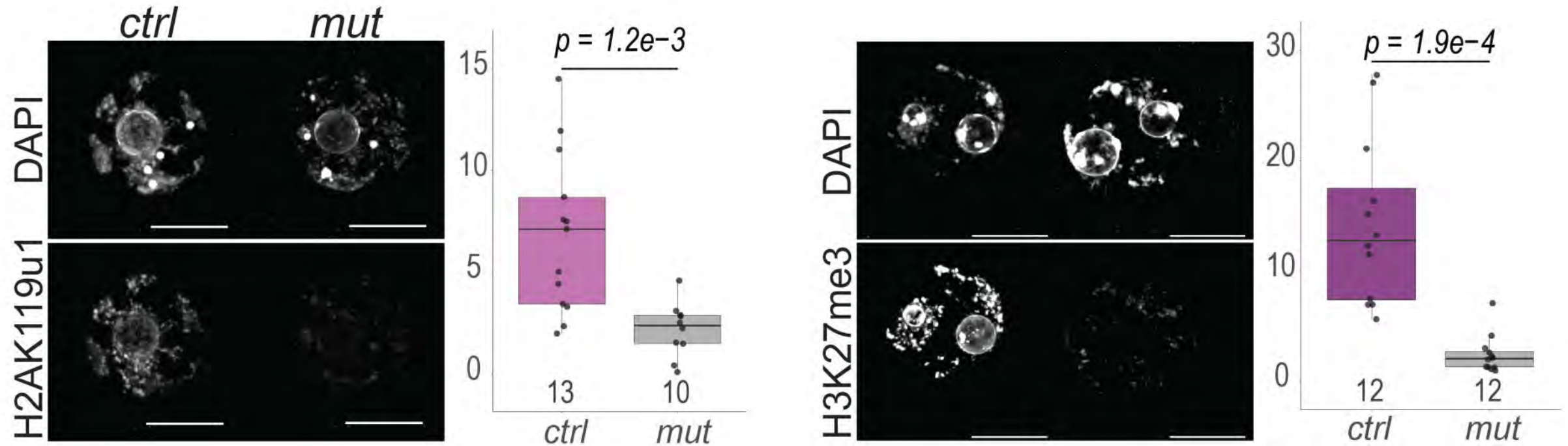
maintenance of silencing



What is the role of KDM2A and KDM2B proteins in oocytes and embryos?

H2AK119u1 and H3K27me3 levels are reduced in *Kdm2a/Kdm2b* mutant oocytes

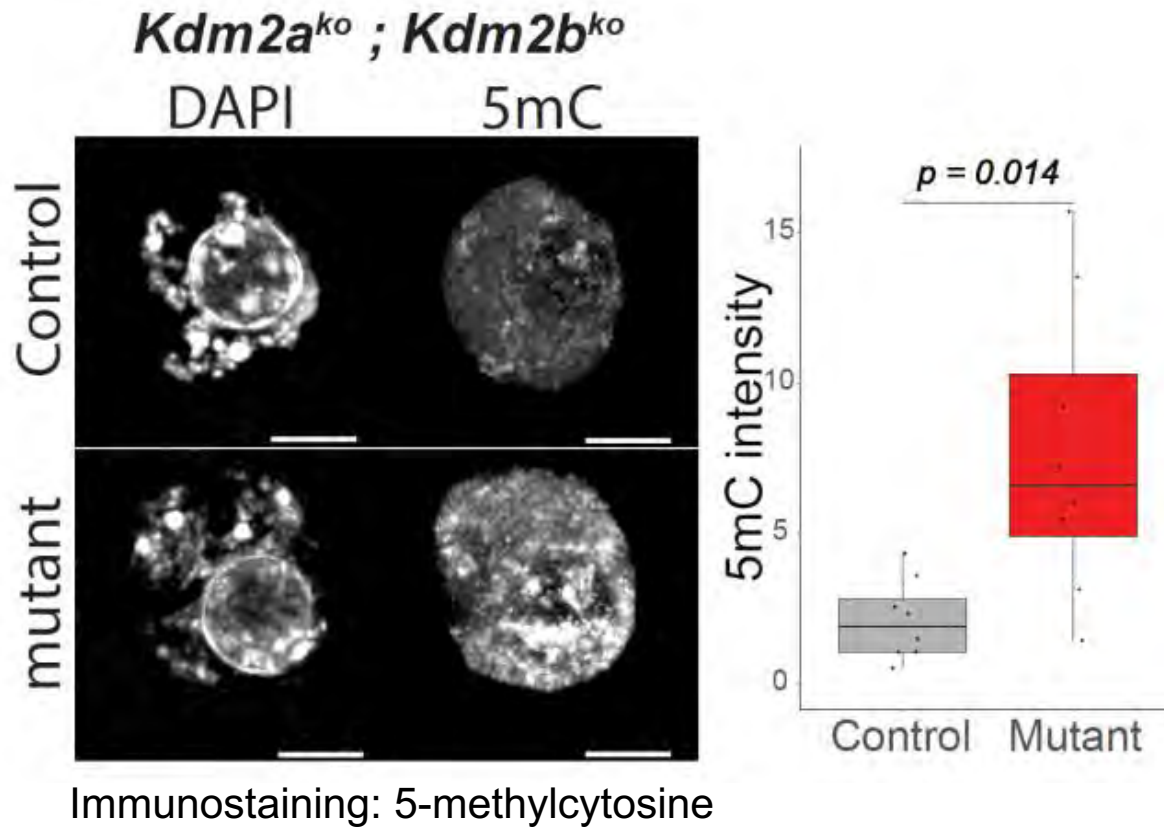
Kdm2a^{ko}; Kdm2b^{ko}



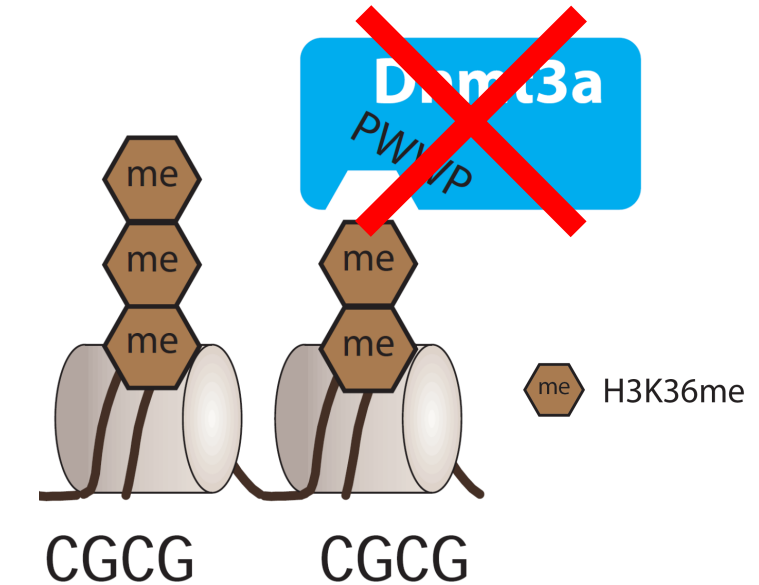
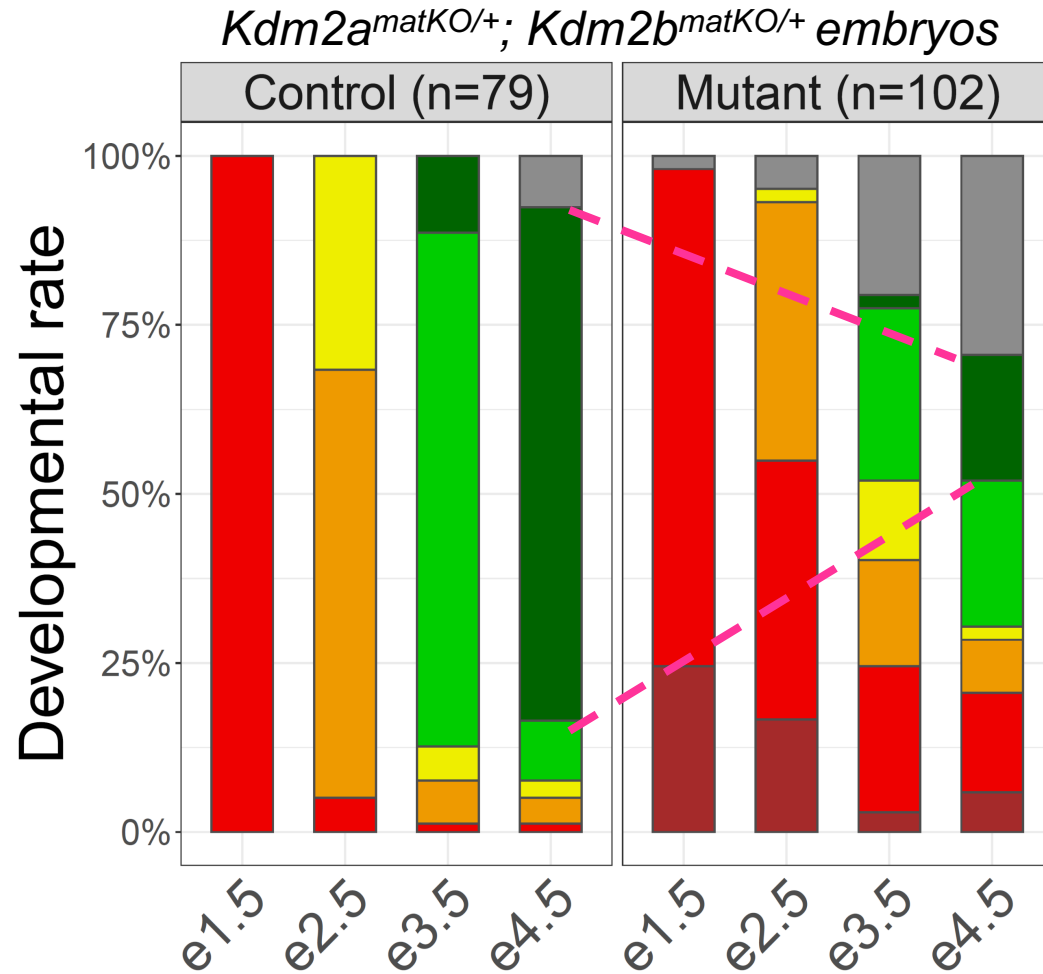
KDM2A/KDM2B recruits PRC1 to chromatin in growing oocytes

PRC1 functions upstream of PRC2

CpG dinucleotide methylation is majorly increased in *Kdm2a/Kdm2b* double mutant oocytes



Does the gain of DNA methylation in oocytes cause developmental defects?

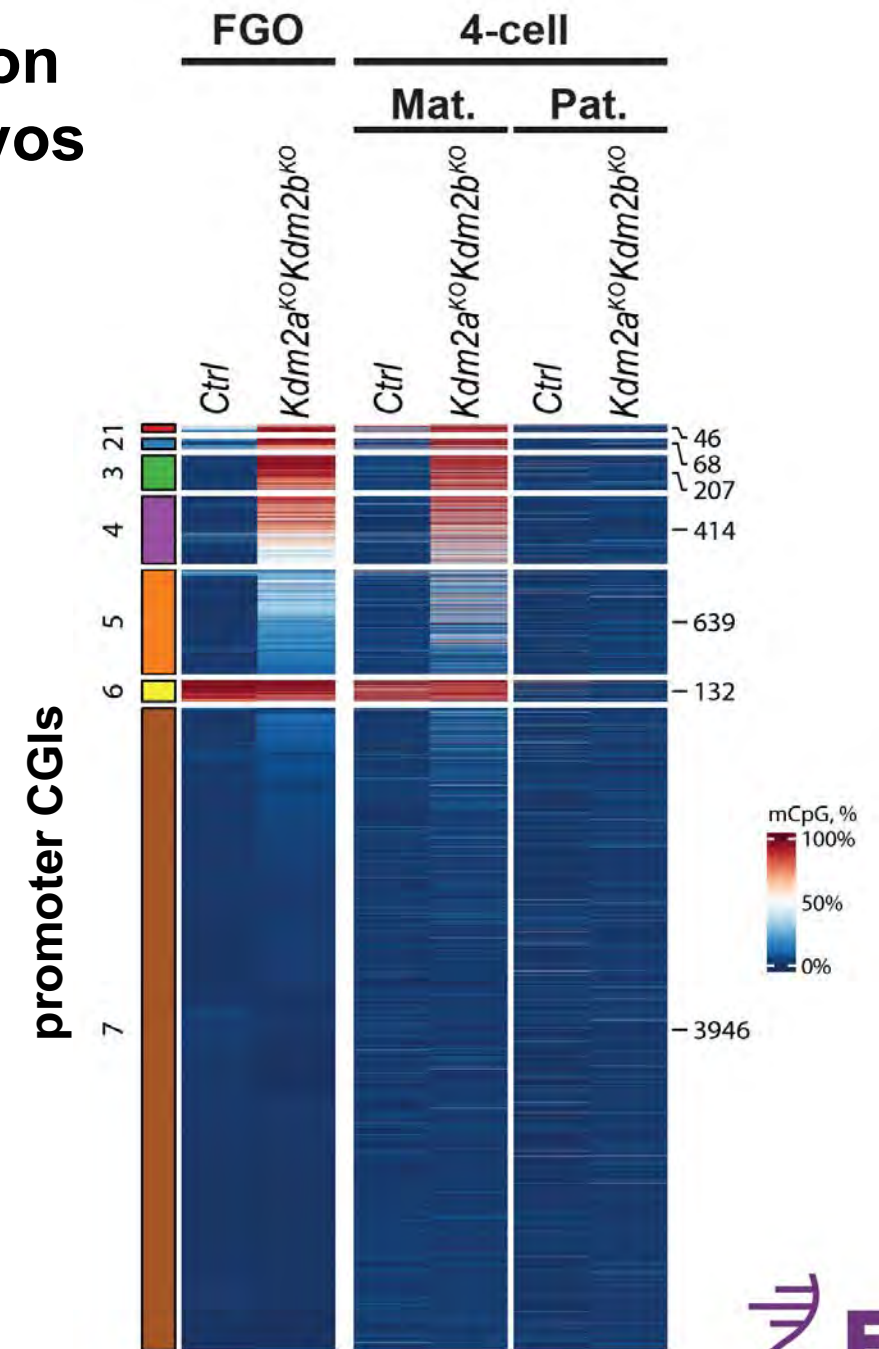
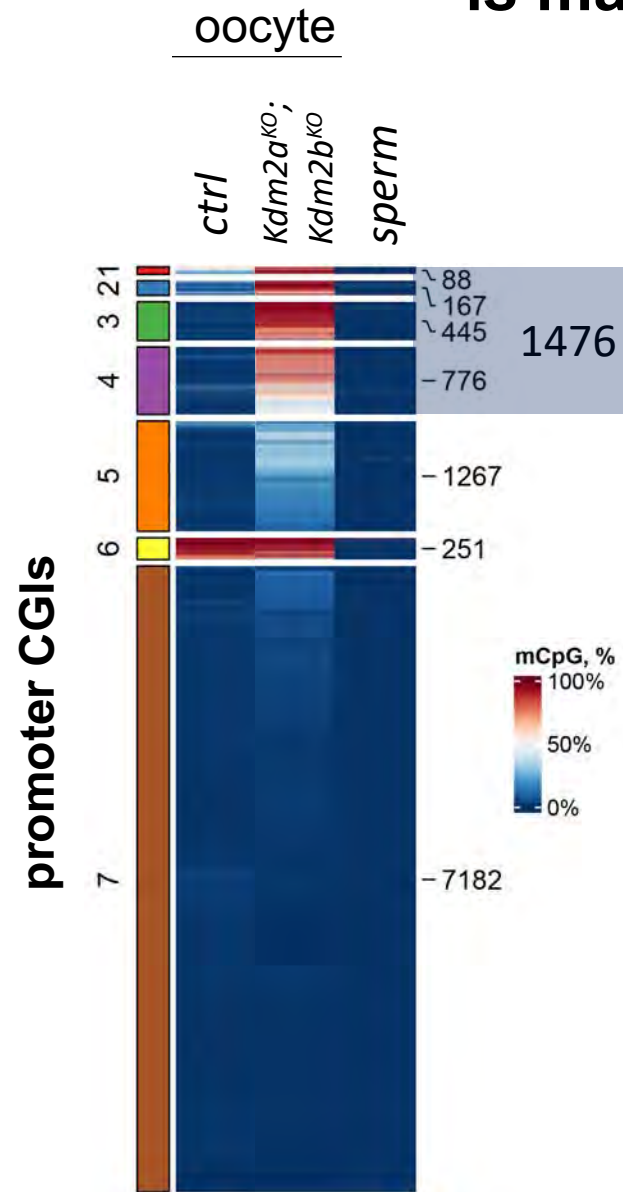


→ Removal of *Dnmt3a* in *Kdm2a/Kdm2b* dko oocytes

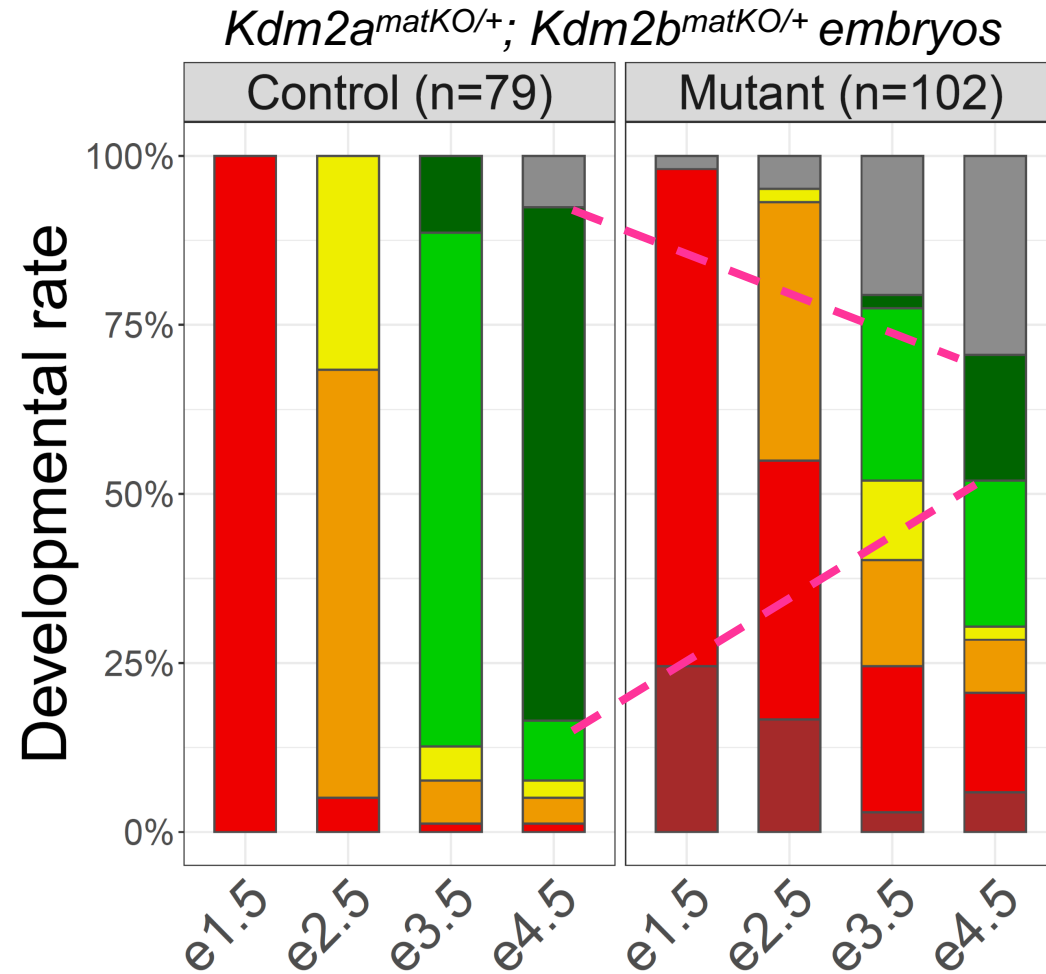
stage

zygote	4/6-cell	morula	fragmented
2/3-cell	8-cell	blastocyst	

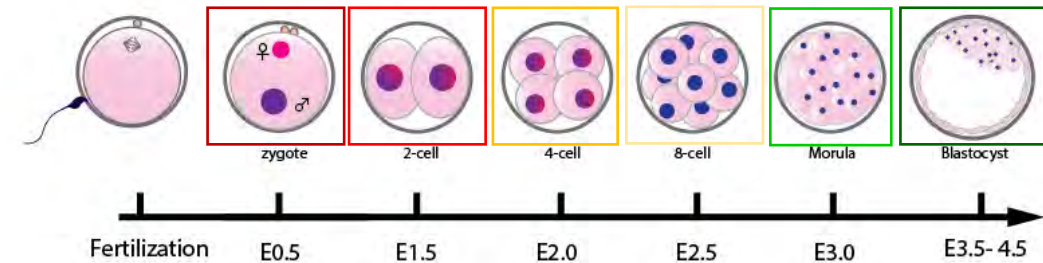
Aberrant maternal DNA methylation is maintained in 4-cell stage embryos



Kdm2a/Kdm2b double deficiency in oocytes impairs embryonic development



mutant oocyte
X
wt sperm

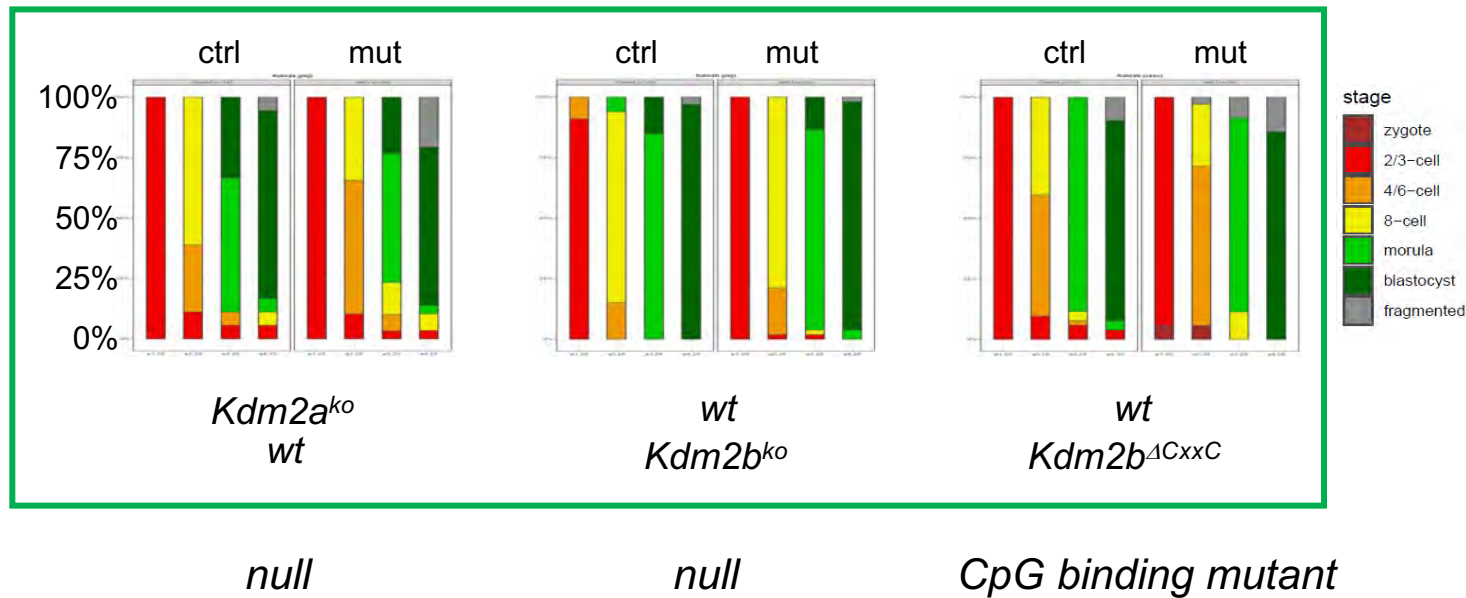
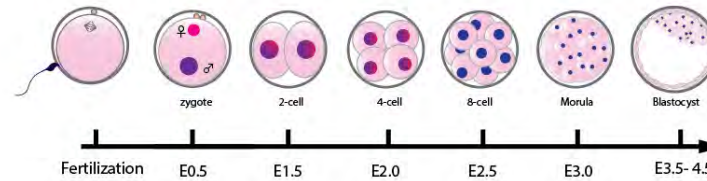


stage

zygote	4/6-cell	morula	fragmented
2/3-cell	8-cell	blastocyst	

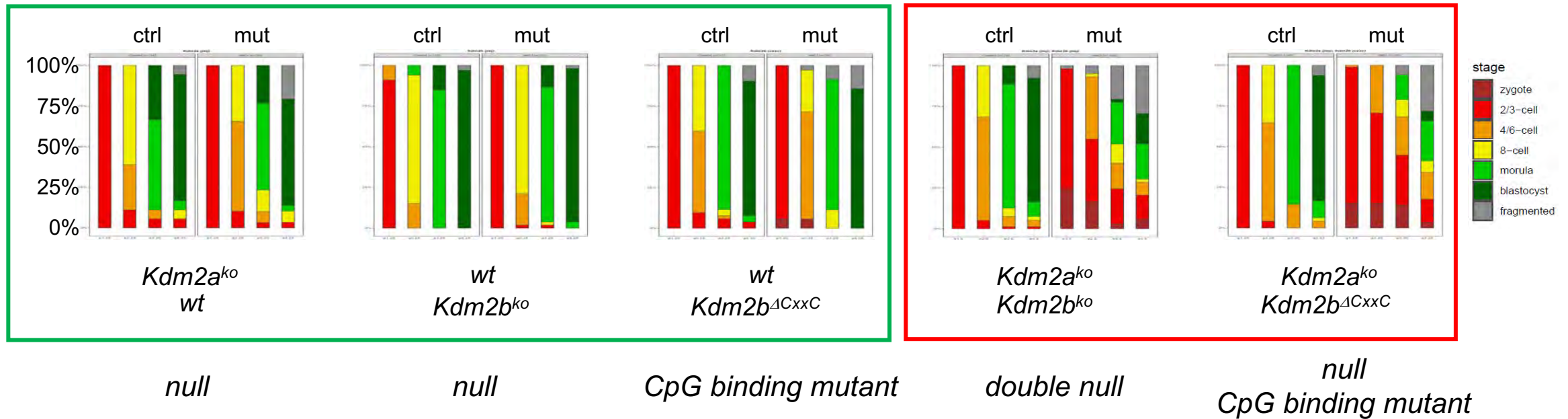
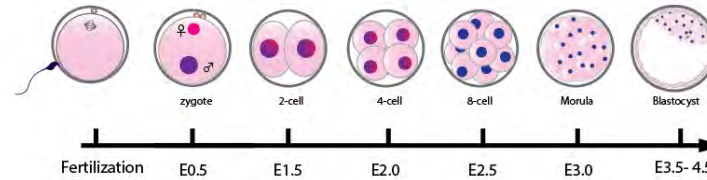
Single *Kdm2a*^{ko}, *Kdm2b*^{ko} and *Kdm2b*^{ΔCxxC} mutations in oocytes do not impair preimplantation development

mutant oocyte x wildtype sperm



Double *Kdm2a/Kdm2b* mutations in oocytes impair preimplantation development

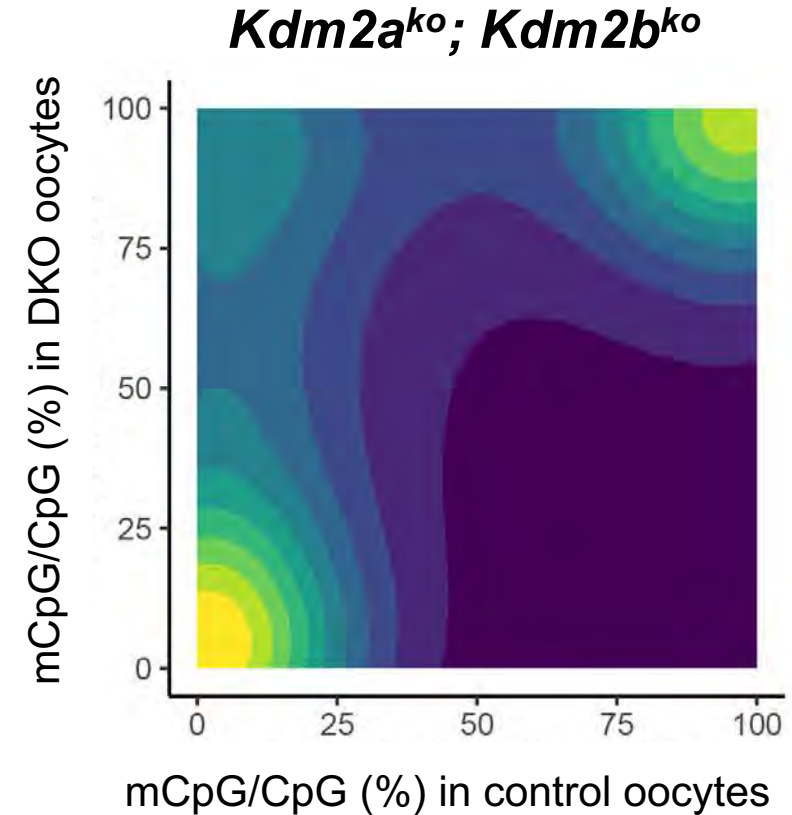
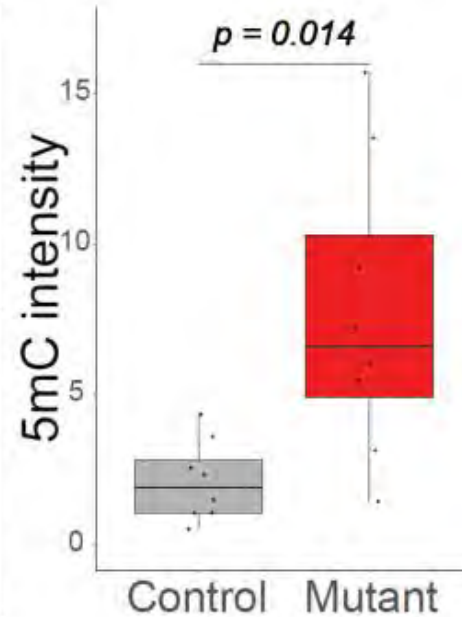
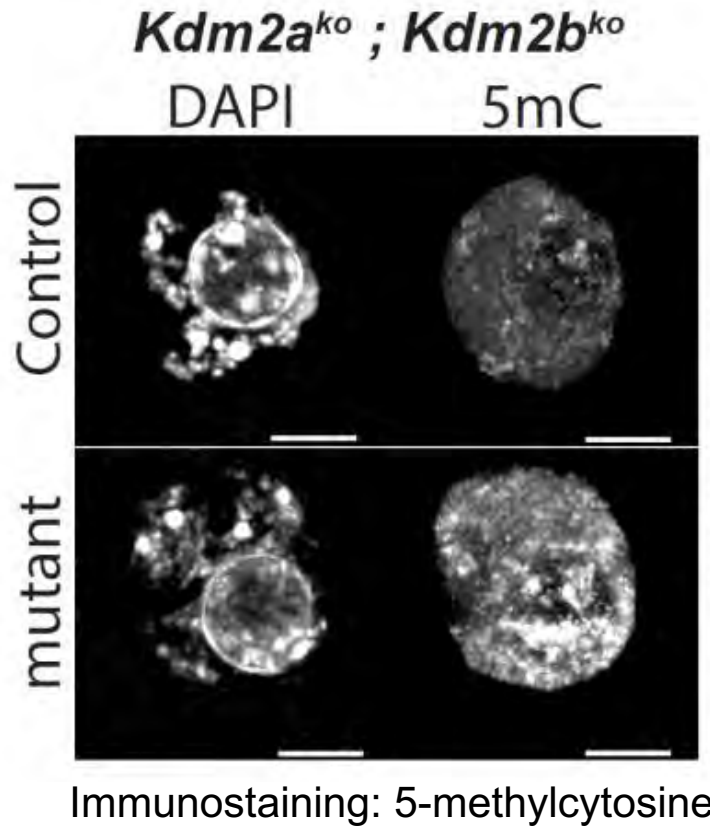
mutant oocyte x wildtype sperm



Normal numbers of oocytes are ovulated after hormonal induction in any of the mutants studied.

Kdm2a and *Kdm2b* fulfill essential “maternal functions” in oocytes
to support embryogenesis

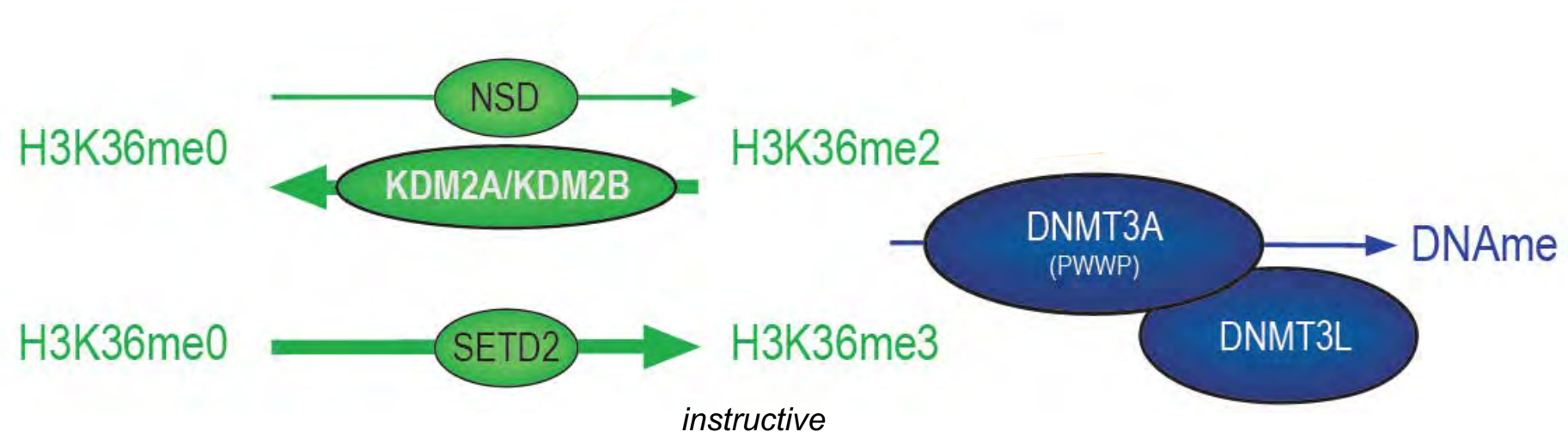
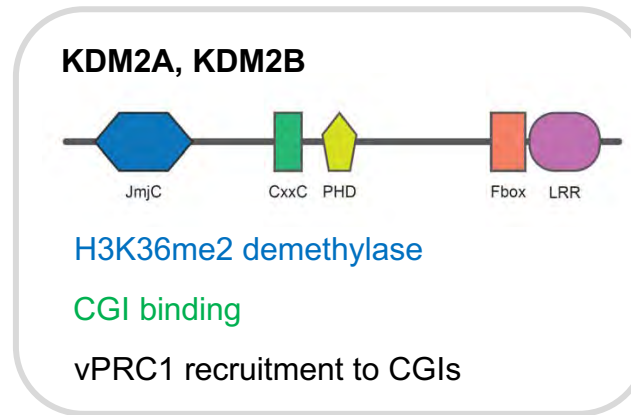
CpG dinucleotide methylation is majorly increased in *Kdm2a/Kdm2b* double mutant oocytes



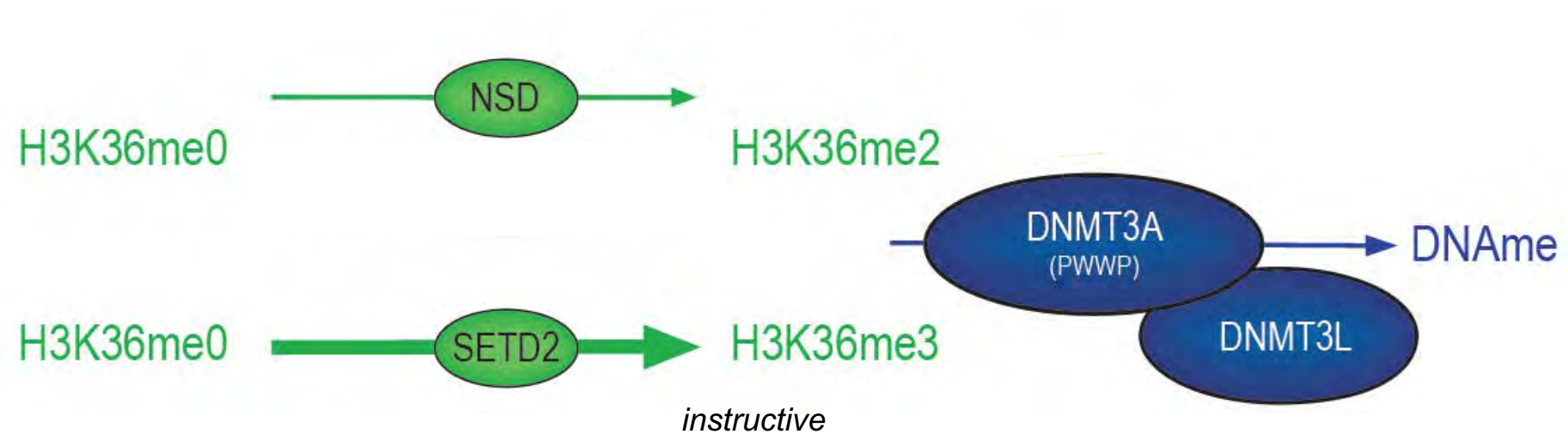
(whole genome bisulfite sequencing)
(130 oocytes / genotype)

WGBS: global gain of methylation: 36% → 59%

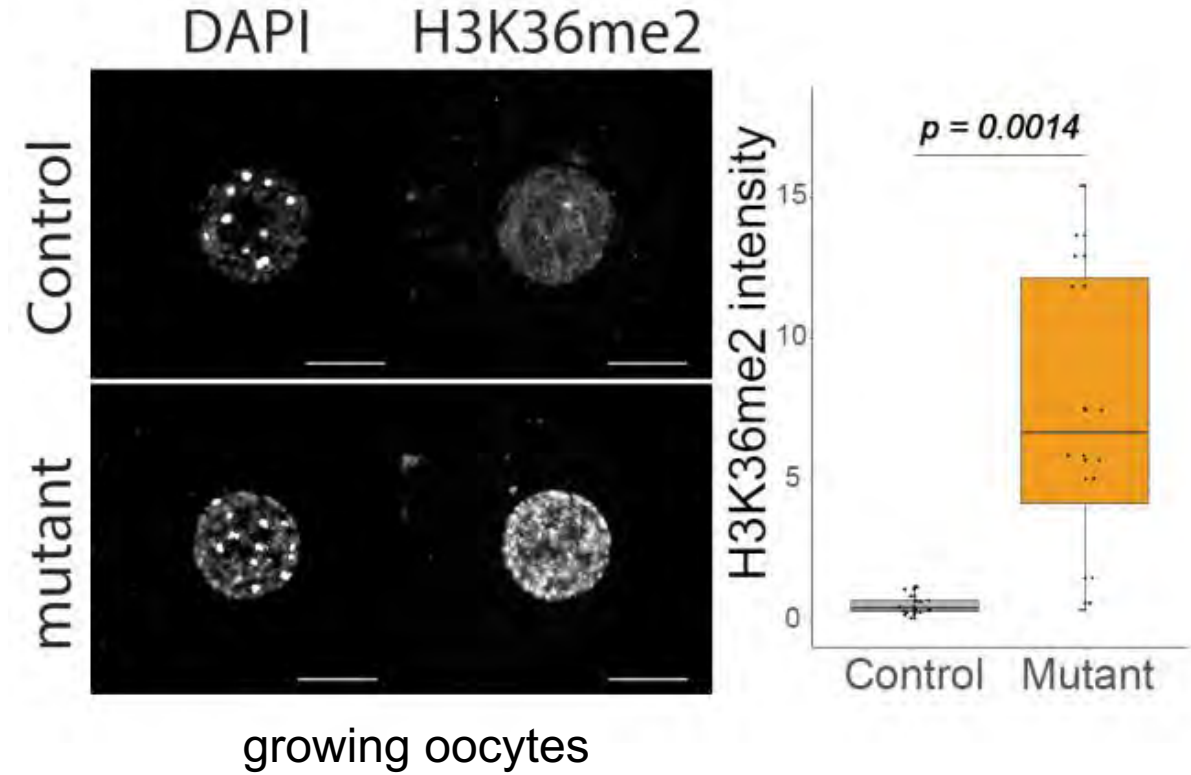
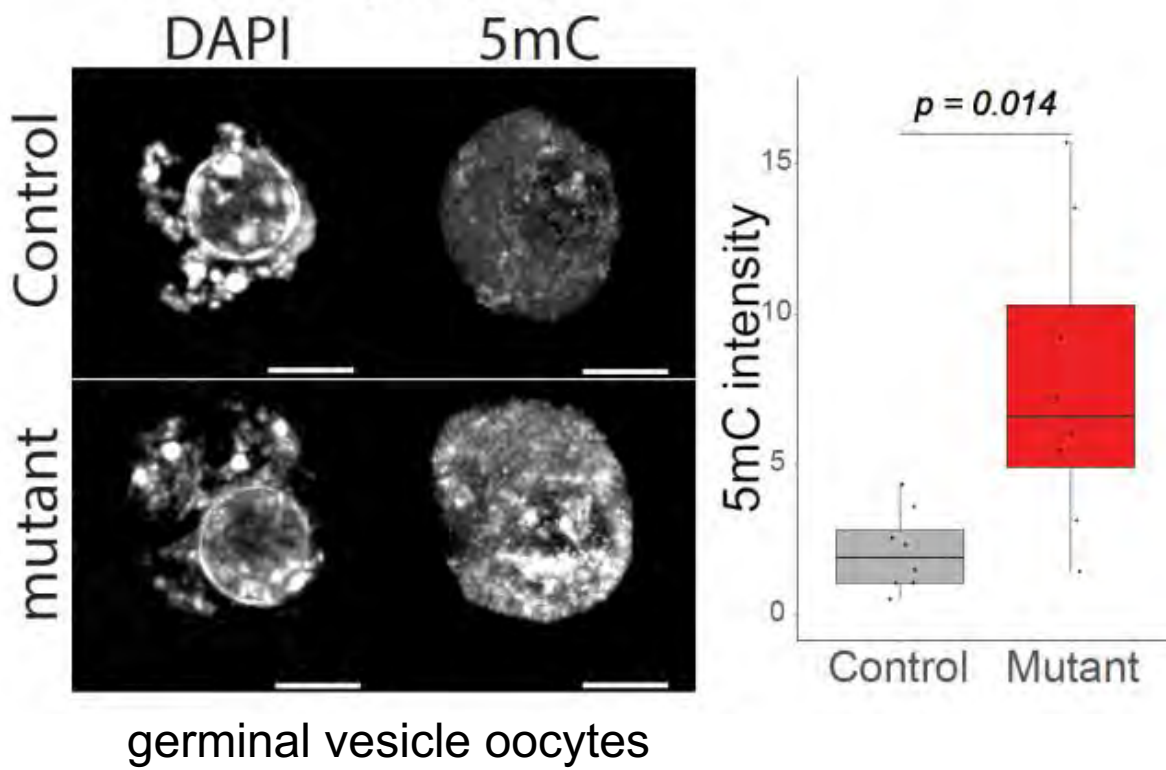
Are KDM2A/KDM2B preventing H3K36me2 accumulation in oocytes?



H3K36 methylation directs *de novo* methylation in somatic and male germ cells

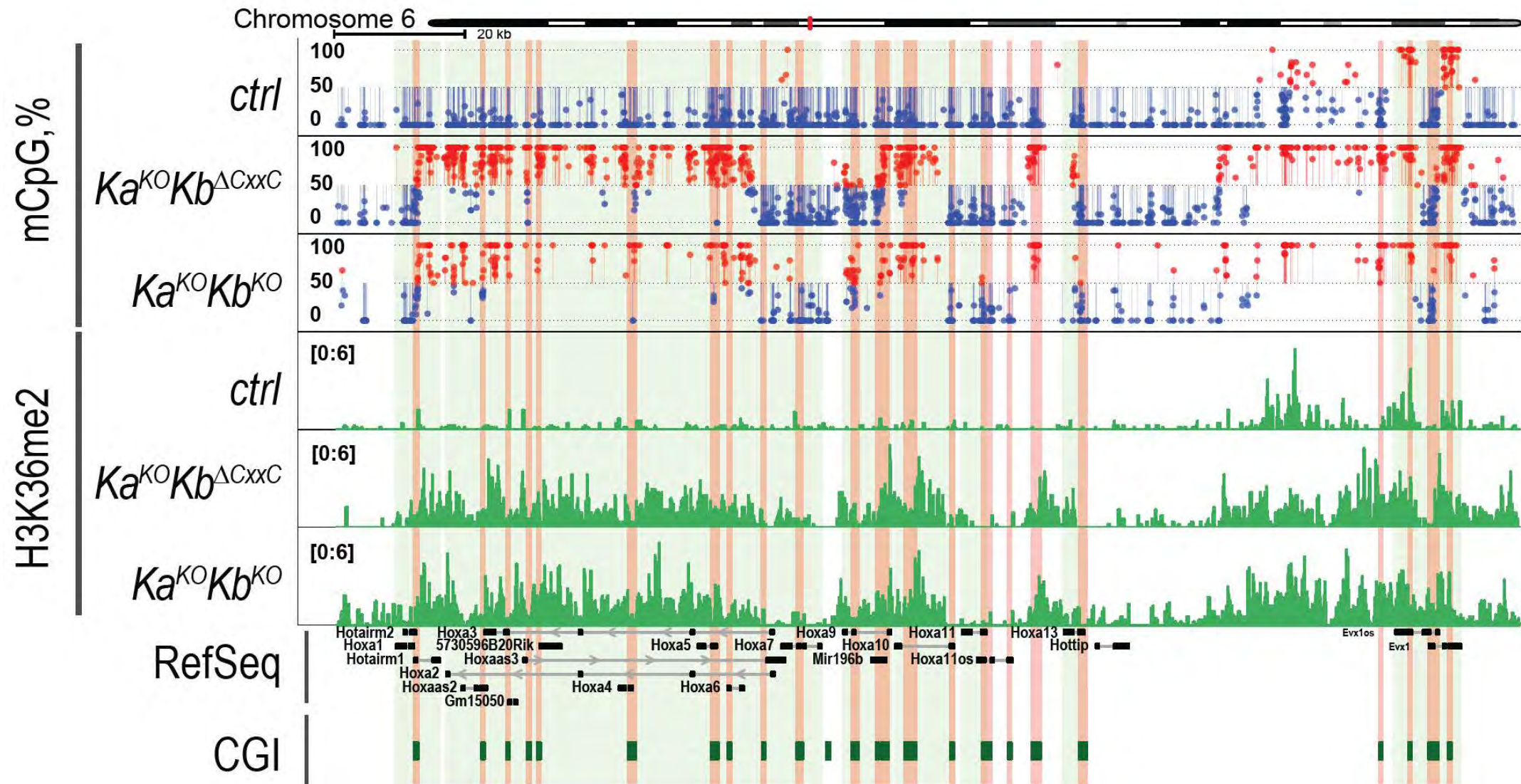


Like CpG methylation, H3K36me2 is majorly increased in *Kdm2a/Kdm2b* double mutant oocytes



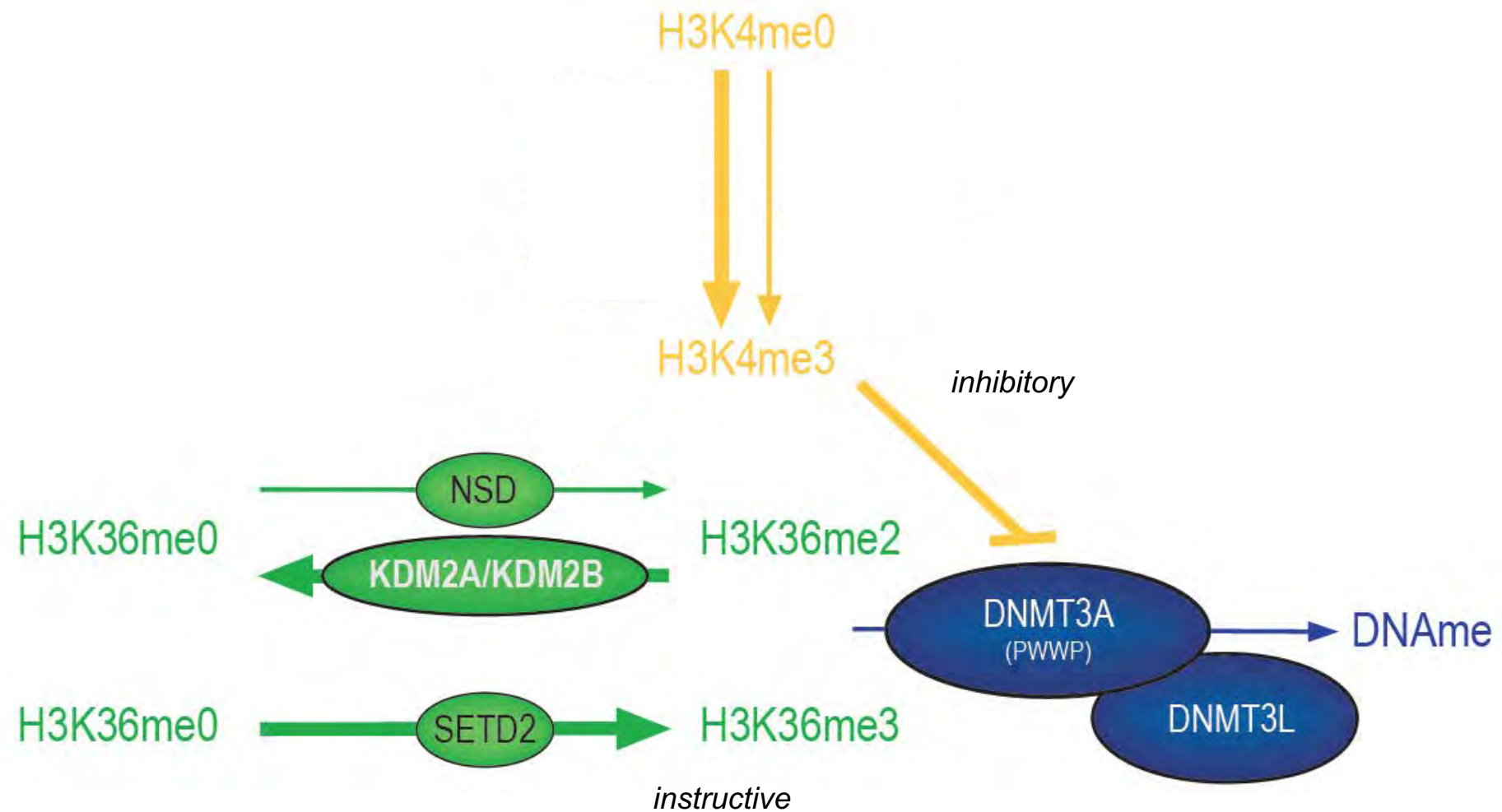
Where is the gain of H3K36me2 ?

Massive gain in H3K36me2 and DNAm in the *Hoxa* gene cluster in *Kdm2a/Kdm2b* double mutant oocytes

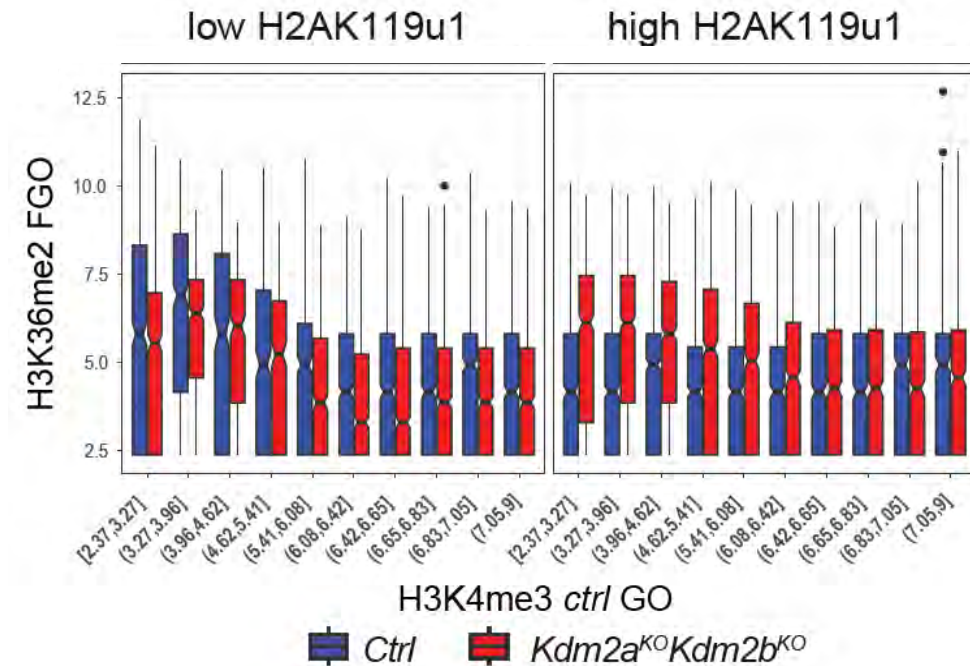
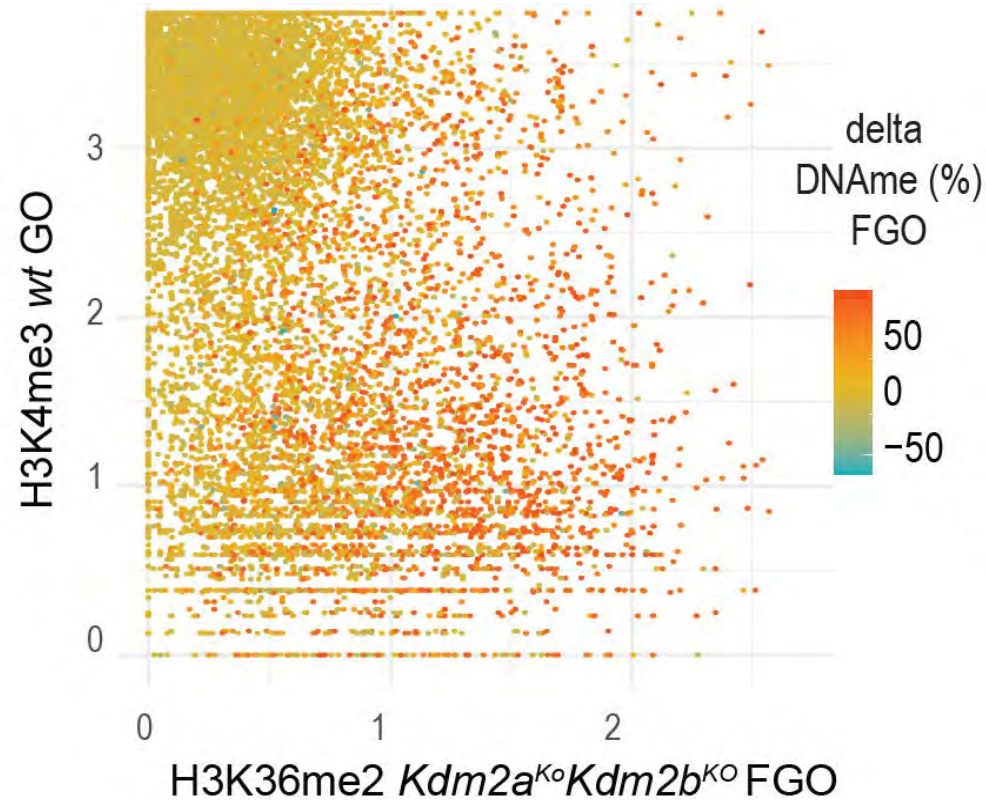


... and barely any gain of H3K36me3

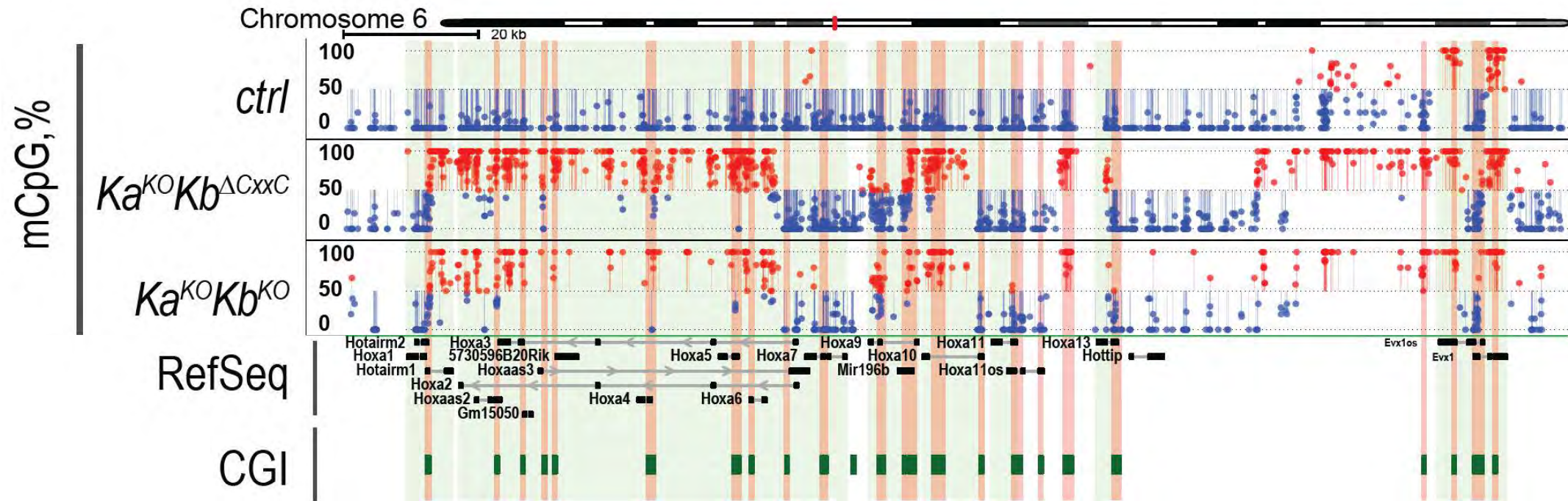
H3K4me3 inhibits DNMT3A activity



H3K4me3 in primary growing oocytes is anti-correlated with H3K36 dimethylation in mutant oocytes

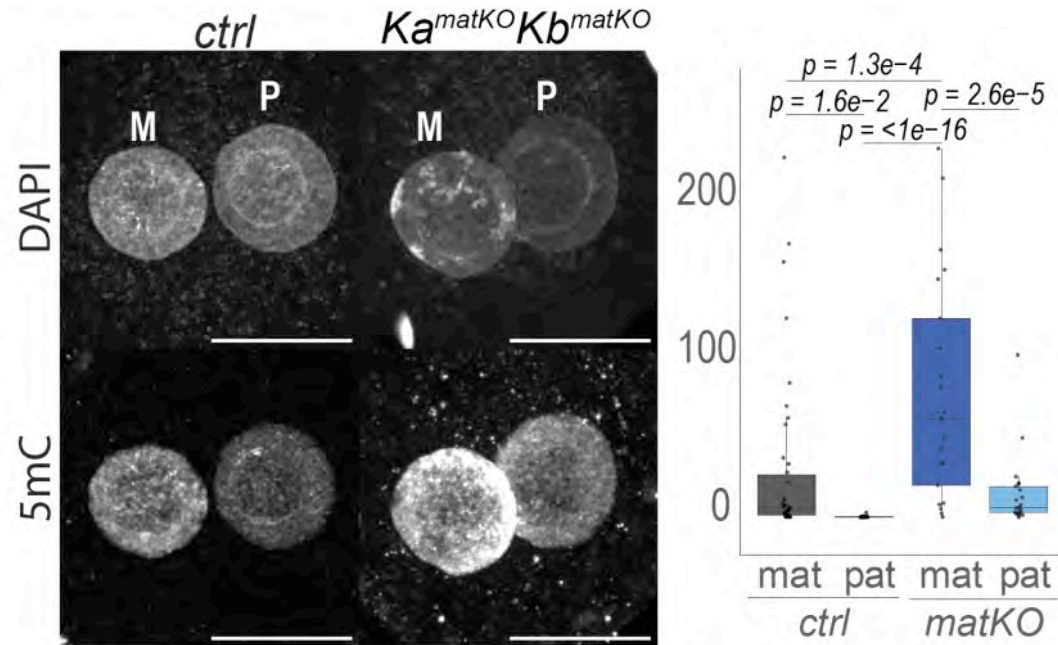


Massive gain in DNAm in the *Hoxa* gene cluster in *Kdm2a/Kdm2b* double mutant oocytes

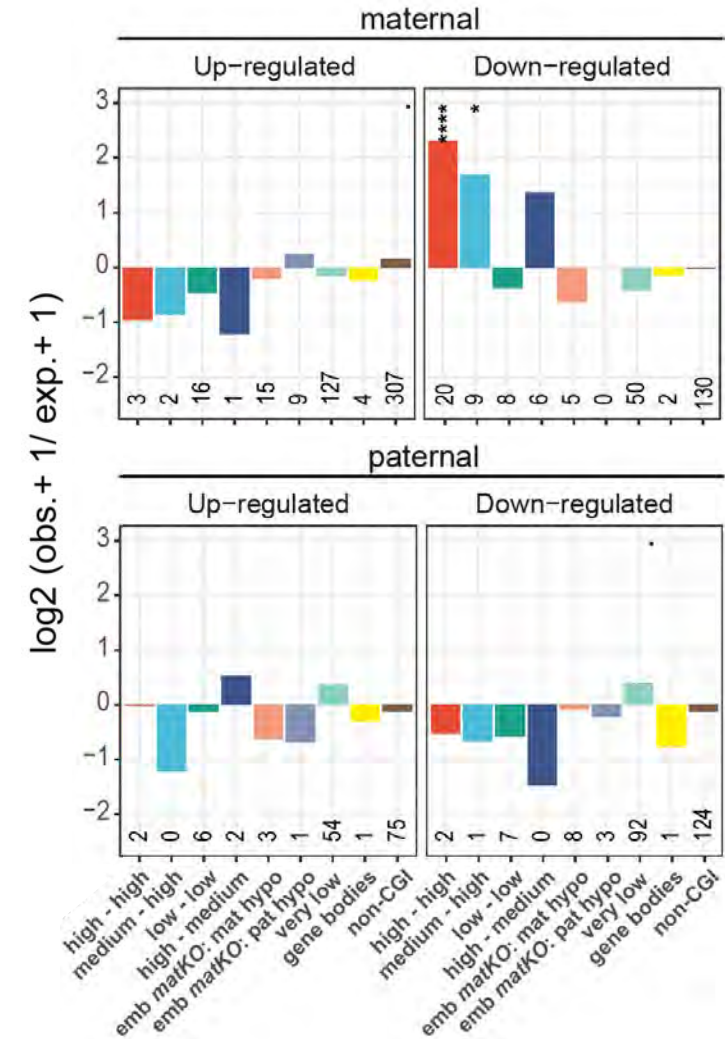
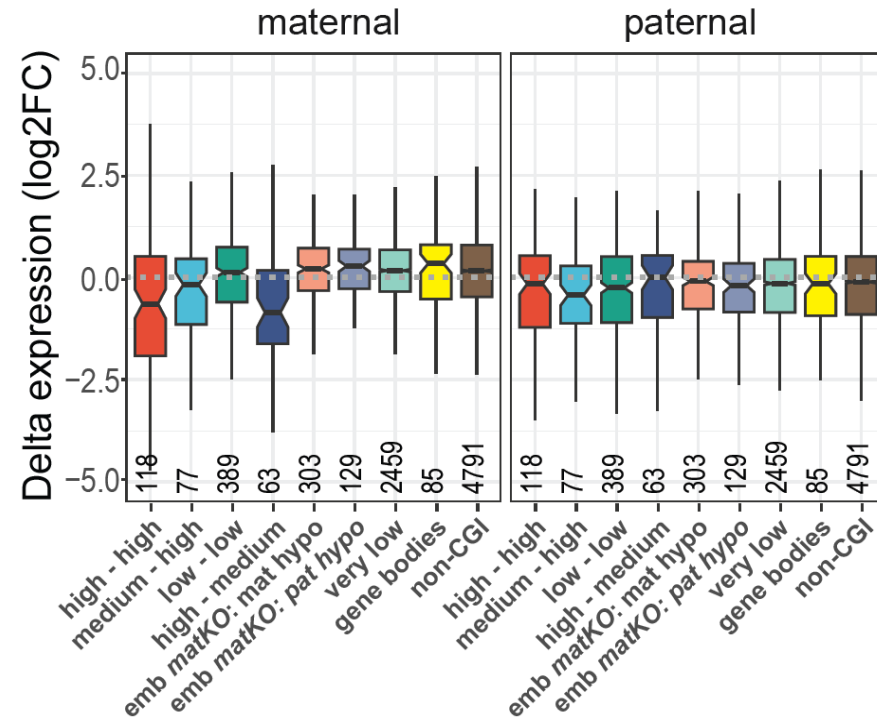
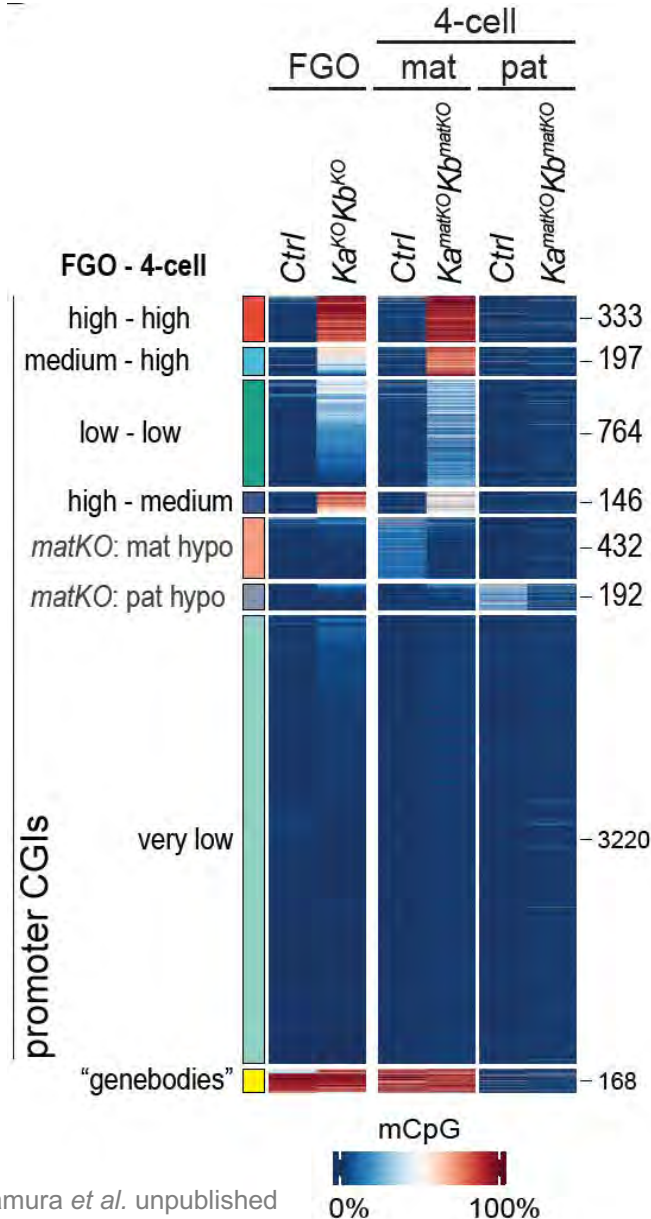


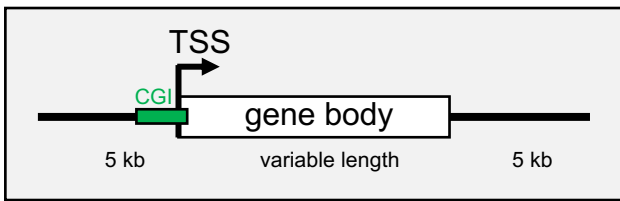
Gain of DNA methylation at transcribed gene bodies,
CpG island promoters and non-transcribed intergenic regions

Aberrant maternal DNA methylation is not reprogrammed in maternally deficient *Kdm2a/Kdm2b* zygote embryos

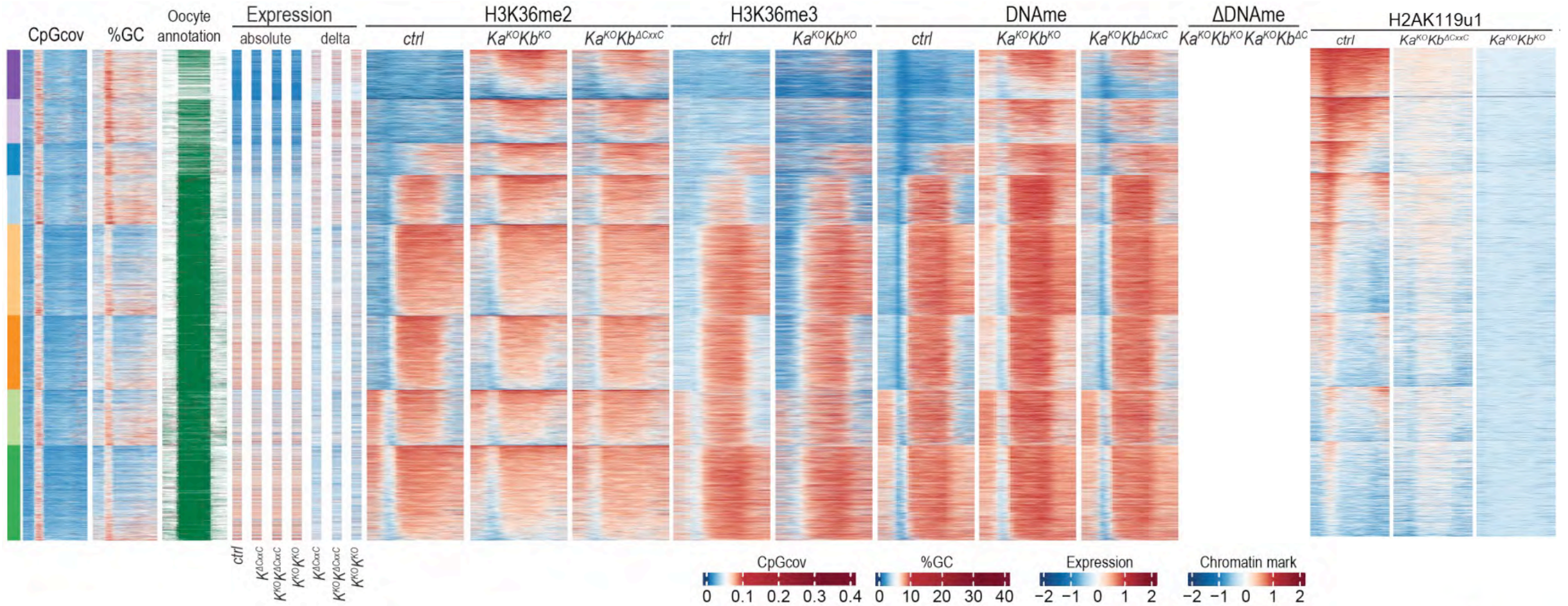


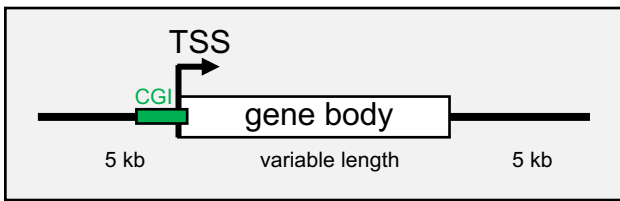
Aberrant DNA methylation associates with gene repression from maternal but not paternal alleles in 2C embryos



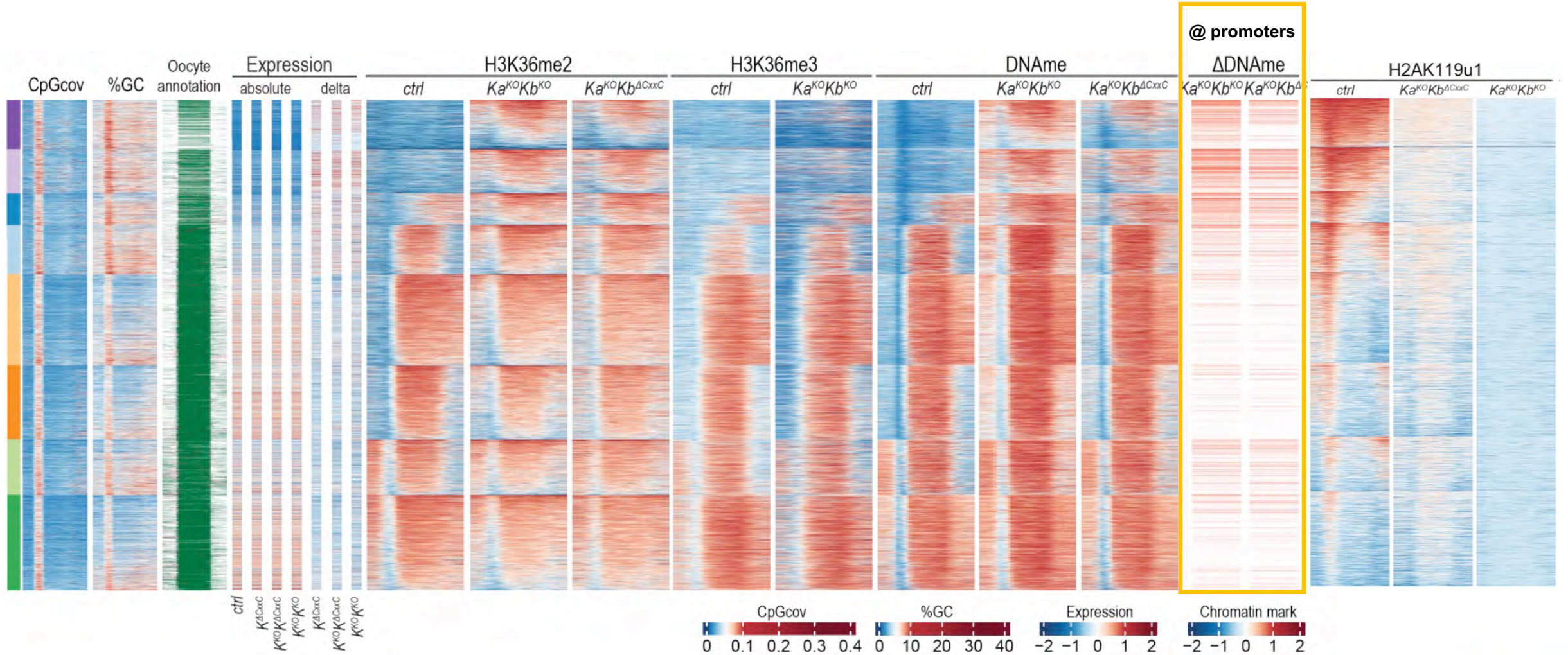


Accumulation of H3K36me2 and DNA methylation at Polycomb-regulated genes in mutant oocytes

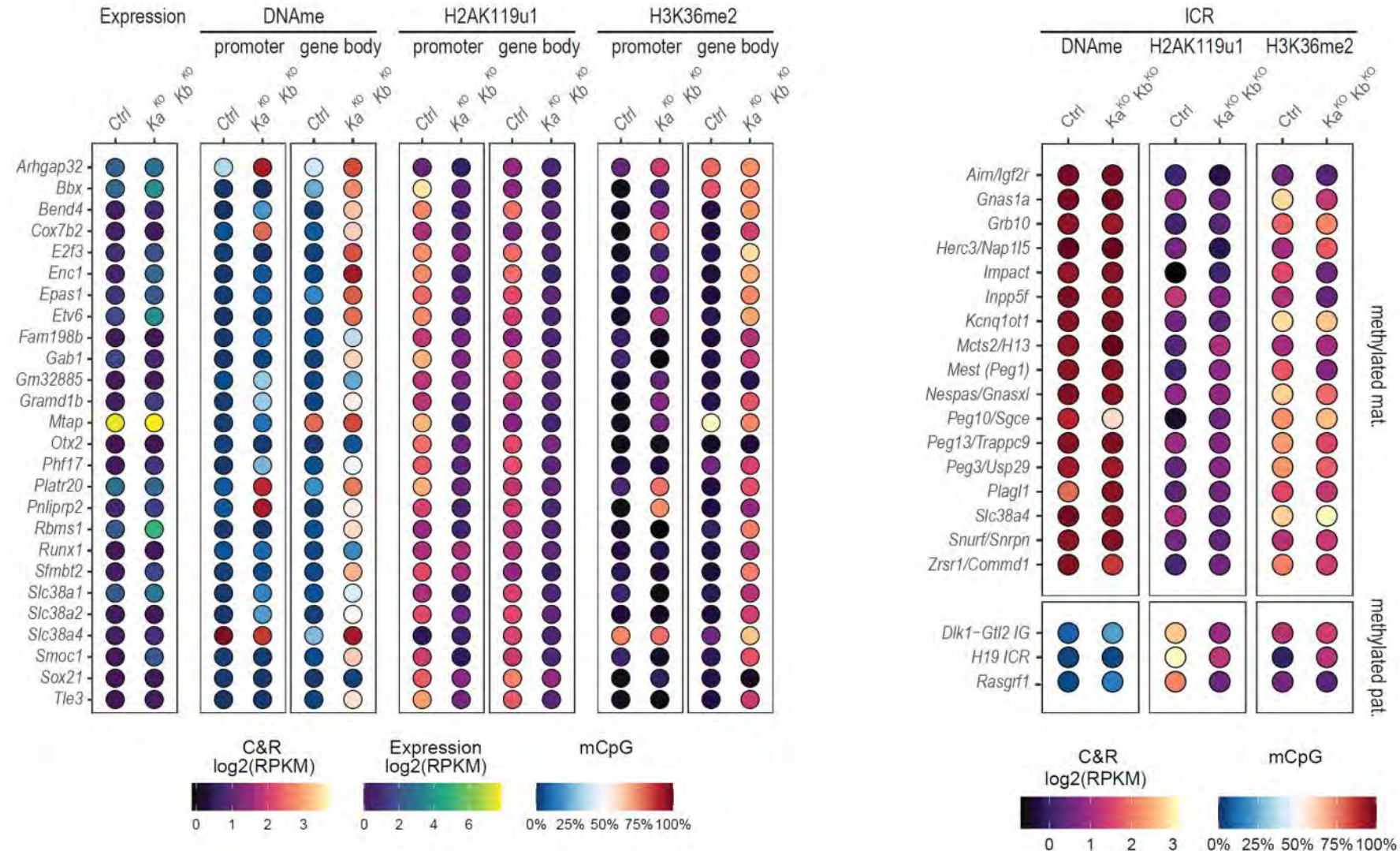




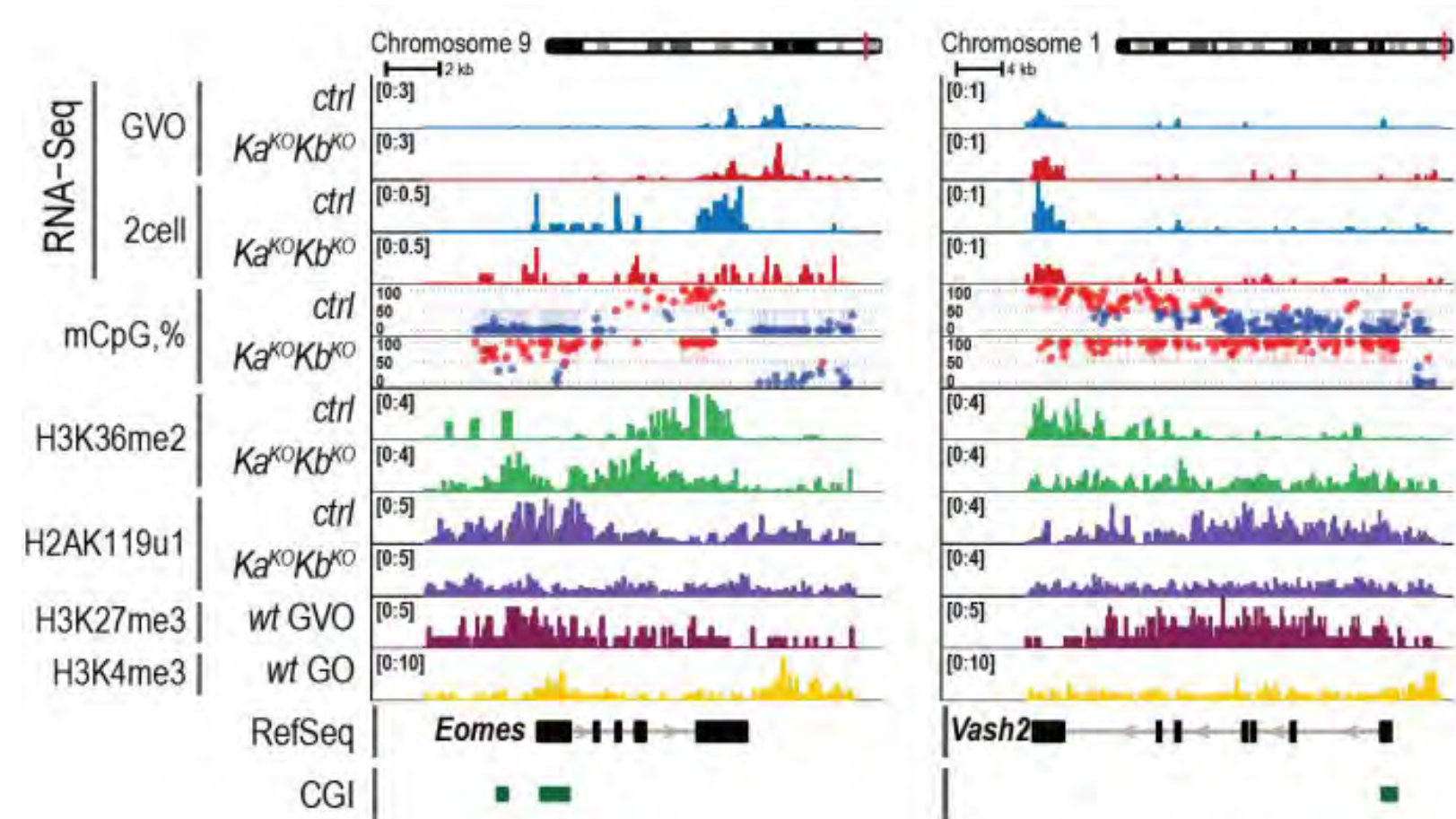
Accumulation of DNA methylation at CGI promoters of PRC1 target and non-target genes in mutant oocytes



Reduction of H2AK119u1 at non-canonically imprinted genes in matDKO oocytes while DNAm at canonical ICRs is unaffected



Outlook: germ line chromatin and early development



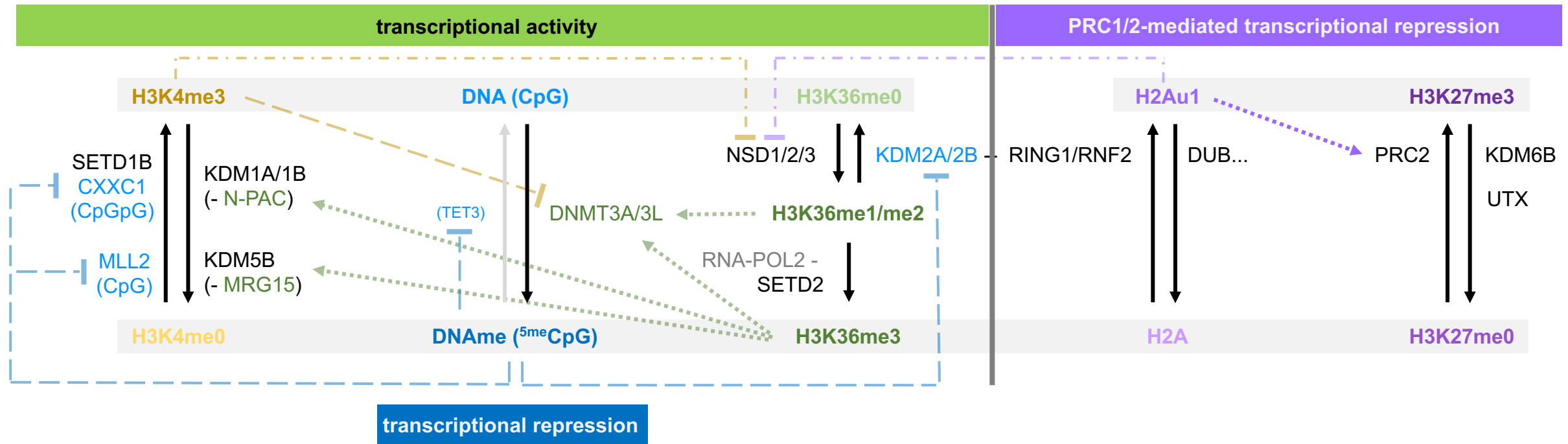
Physiological relevance for embryo and placental development, and in disease?

VASH2: preeclampsia

Mechanisms of DNAm inheritance?

(resistance to reprogramming during pre-implantation development)

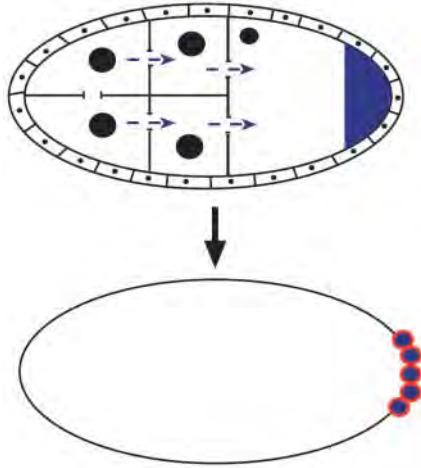
Chromatin network interactions controlling DNA methylation levels at CpG-rich sequences, including CpG islands at promoters and in gene bodies



The origin of germ cells

Preformation

(Proteins, mRNAs)

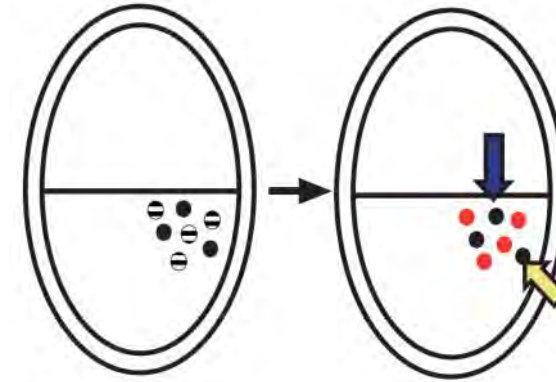


anti-VASA

Drosophila melanogaster

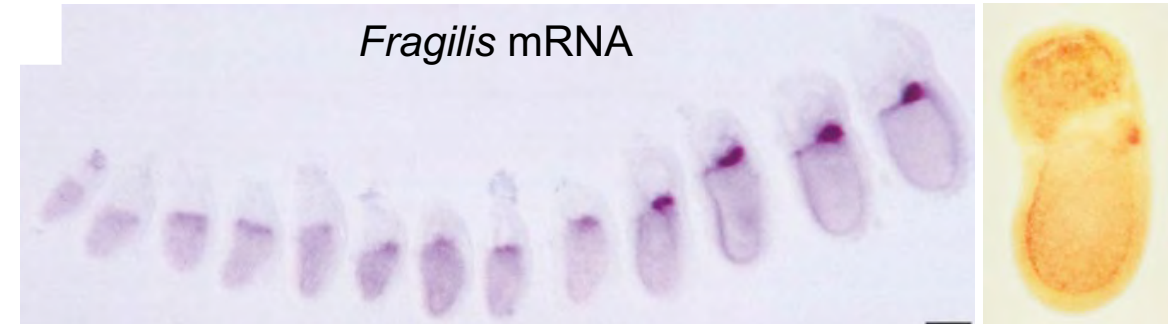
Epigenese

(Signal transduction, Transcription)



*primordial
germ cells*

Alkaline
Phosphatase



Fragilis mRNA

Mouse

HUMAN DEVELOPMENT

TECHNIQUES AND RESOURCES ARTICLE

Spermatogonial kinetics in humans

Sara Di Persio¹, Rossana Saracino¹, Stefania Fera¹, Barbara Muciaccia¹, Valentina Esposito¹, Carla Boitani¹, Bartolomeo P. Berloco², Francesco Nudo², Gustavo Spadetta³, Mario Stefanini¹, Dirk G. de Rooij^{1,*} and Elena Vicini^{1,*}

ABSTRACT

The human spermatogonial compartment is essential for daily production of millions of sperm. Despite this crucial role, the molecular signature, kinetic behavior and regulation of human spermatogonia are poorly understood. Using human testis biopsies with normal spermatogenesis and by studying marker protein expression, we have identified for the first time different subpopulations of spermatogonia. MAGE-A4 marks all spermatogonia, KIT marks all B spermatogonia and UCLH1 all Apale-dark (Ap-d) spermatogonia. We suggest that at the start of the spermatogenic lineage there are Ap-d spermatogonia that are GFRA1^{High}, likely including the spermatogonial stem cells. Next, UTF1 becomes expressed, cells become quiescent and GFRA1 expression decreases. Finally, GFRA1 expression is lost and subsequently cells differentiate into B spermatogonia, losing UTF1 and acquiring KIT expression. Strikingly, most human Ap-d spermatogonia are out of the cell cycle and even differentiating type B spermatogonial proliferation is restricted. A novel scheme for human spermatogonial development is proposed that will facilitate further research in this field, the understanding of cases of infertility and the development of methods to increase sperm output.

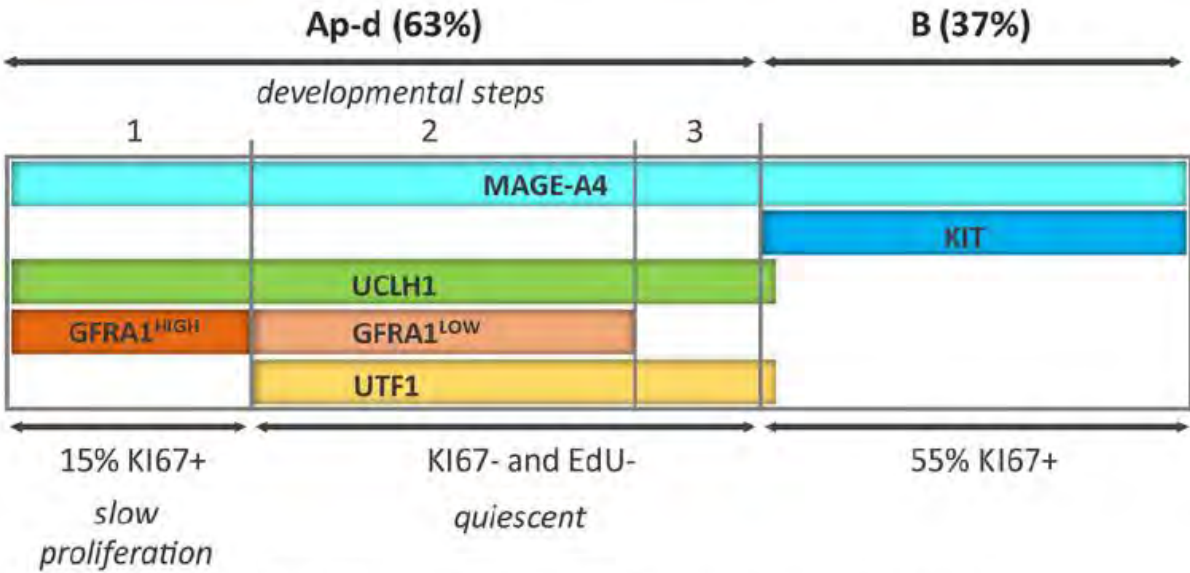
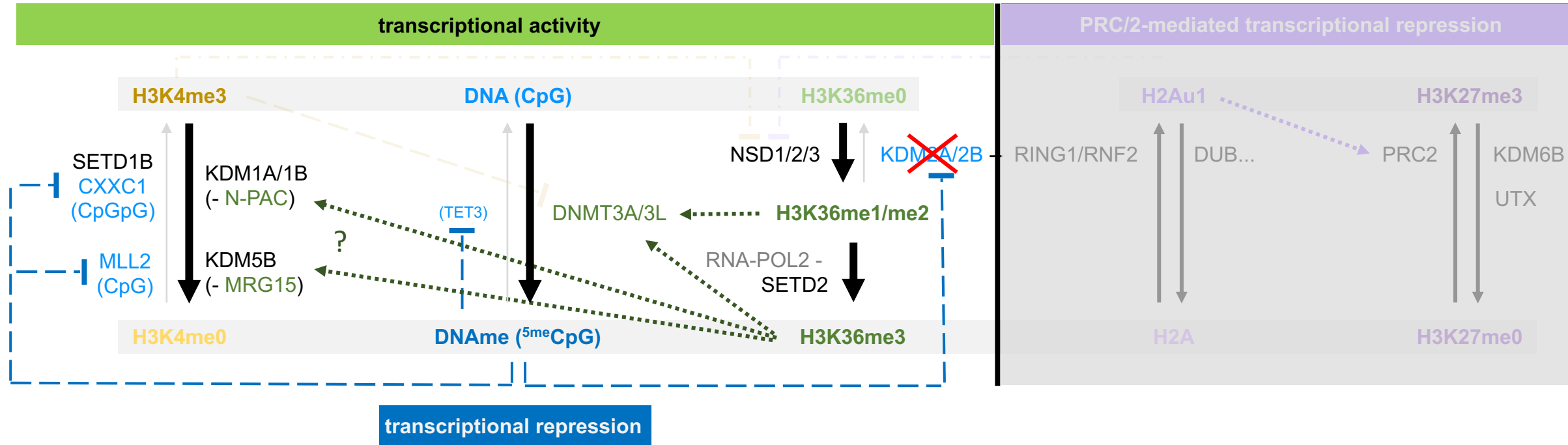


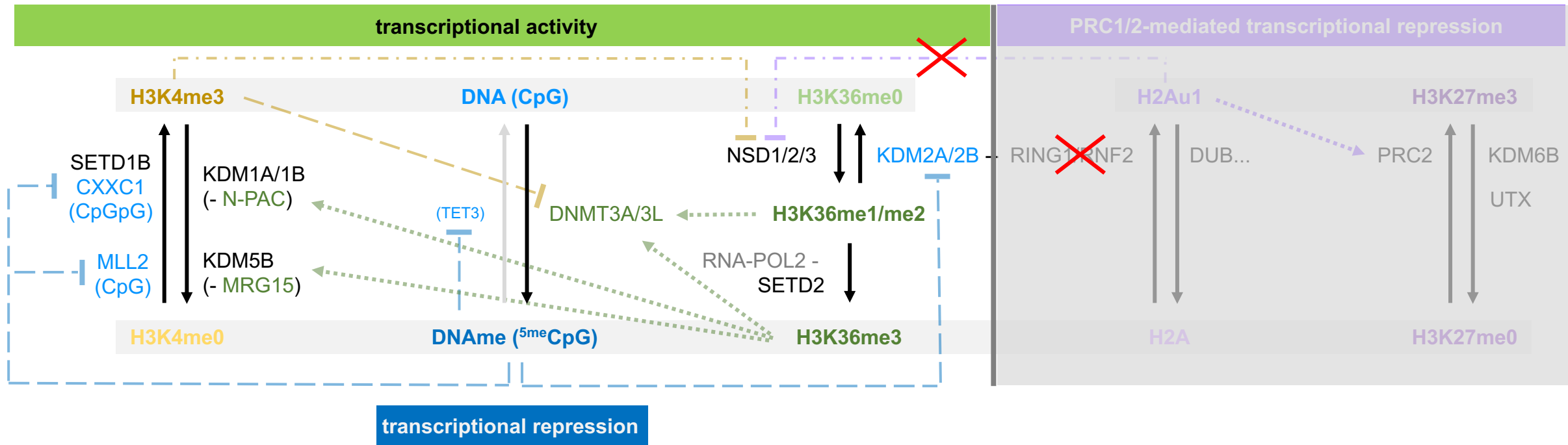
Fig. 6. Proposed scheme for human spermatogonial multiplication and stem cell renewal, based on marker protein expression and mitotic activity of the cells. The SSCs reside in the GFRA1^{High}/UTF1⁻ Ap-d spermatogonia and differentiating cells are recruited from GFRA1^{Low}/UTF1⁺ Ap-d spermatogonia. Note the very low proliferative activity of the Ap-d.

Loss of KDM2A/KDM2B (and RING/RNF2) function: gain of DNA methylation at CpG-rich sequences

promoter

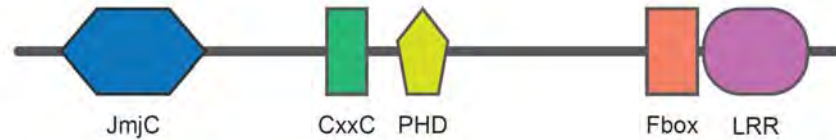


Loss of RING1/RNF2 function: no gain of DNA methylation at CpG-rich sequences, suggesting normal KDM2A/KDM2B function

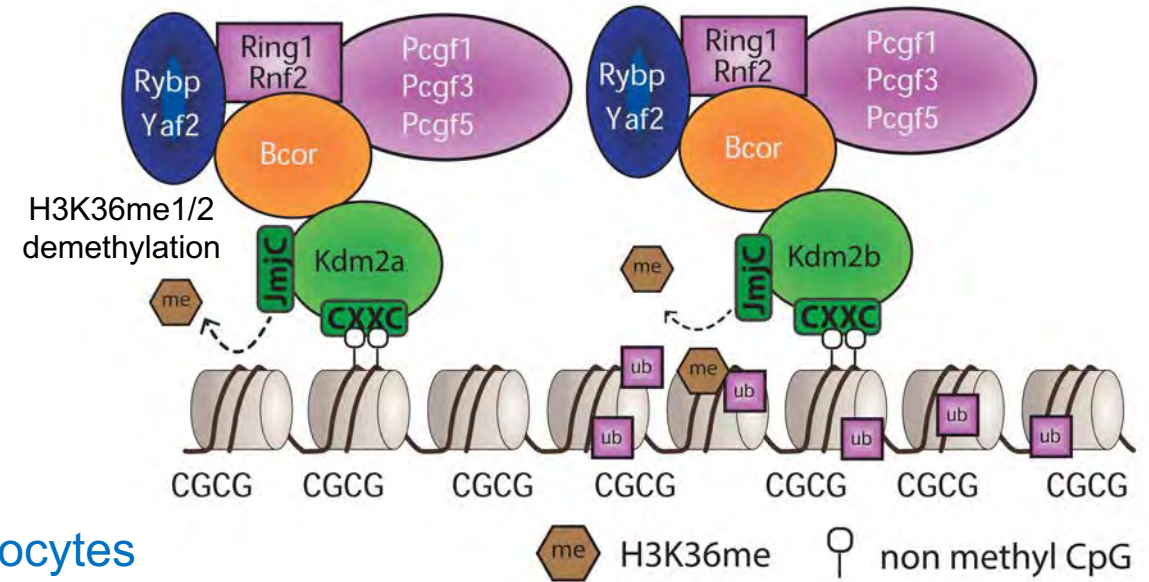


Conditional deficiency study of *Kdm2a* and *Kdm2b* function in oocytes

protein domain structure



in mouse oocytes



- *Kdm2a* and *Kdm2b* are both expressed in growing oocytes
- Mutations: *Kdm2a* floxed JmjC → KO / null allele
- Kdm2b* floxed JmjC → KO / null allele
- Kdm2b* floxed CxxC → in frame deletion; no recruitment to unmethylated CpGs

Takashi Kondo & Haruhiko Koseki

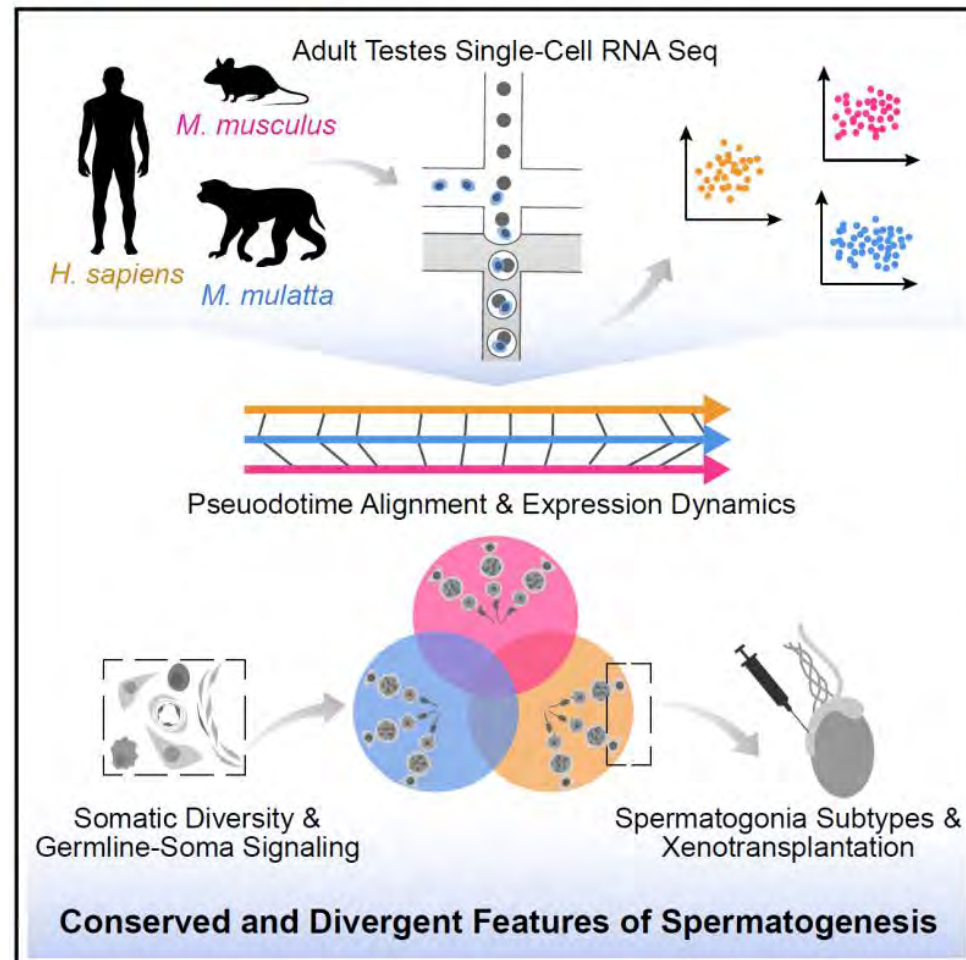
Laboratory for Developmental Genetics, RIKEN, Yokohama, Japan



Developmental Cell

Single-Cell RNA Sequencing of Human, Macaque, and Mouse Testes Uncovers Conserved and Divergent Features of Mammalian Spermatogenesis

Graphical Abstract



Authors

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In Brief

Spermatogenesis has been studied extensively in rodents, but the conservation among species is not well understood. Shami et al. employ single-cell RNA sequencing to compare transcriptional signatures of thousands of testes cells from human, macaque, and mouse, creating a resource to advance understanding of mammalian reproduction and evolution.

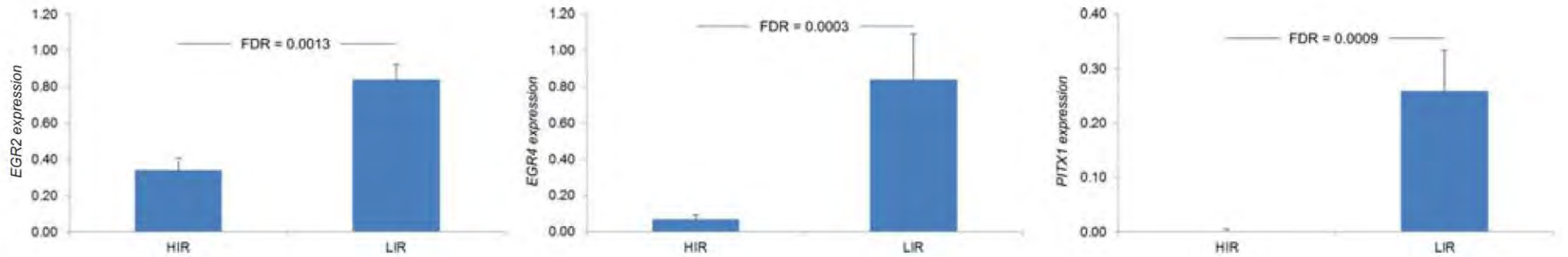
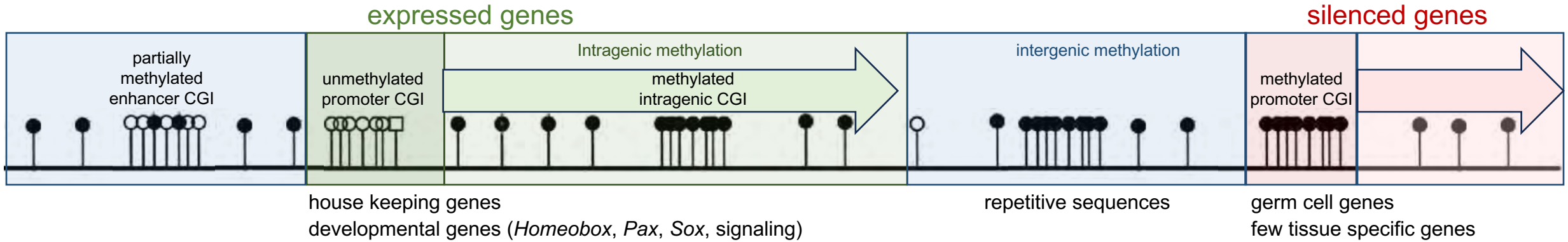
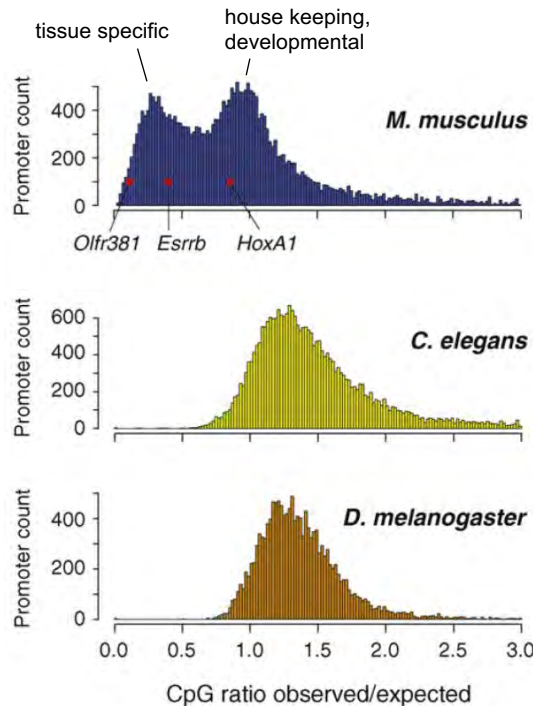
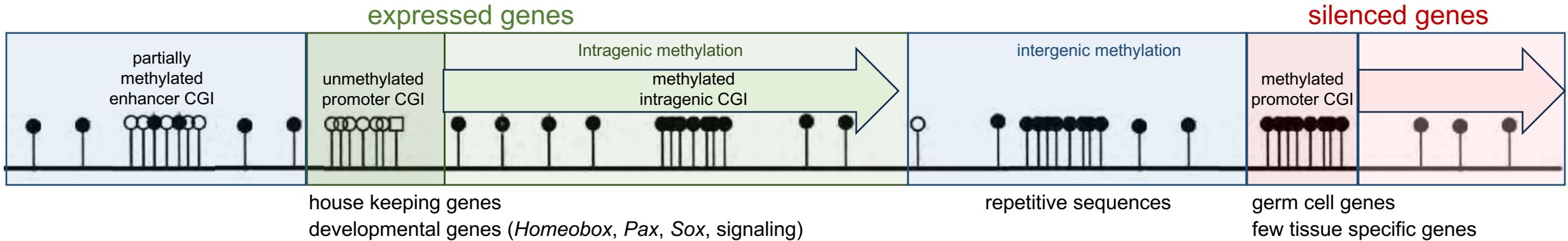


Fig. 2. Expression levels of *EGR2*, *EGR4*, and *PITX1* analyzed by RNA sequencing in HIR and LIR cryptorchid testes. Gene expression shown as median RPKM values, mean absolute deviations and FDR values is presented.

CpG dinucleotides are methylated throughout the genome in mammalian somatic cells except for most CpG islands (CGIs)



CpG dinucleotides are methylated throughout the genome in mammalian somatic cells except for most CpG islands (CGIs)



Cytosine to Thymine transition:

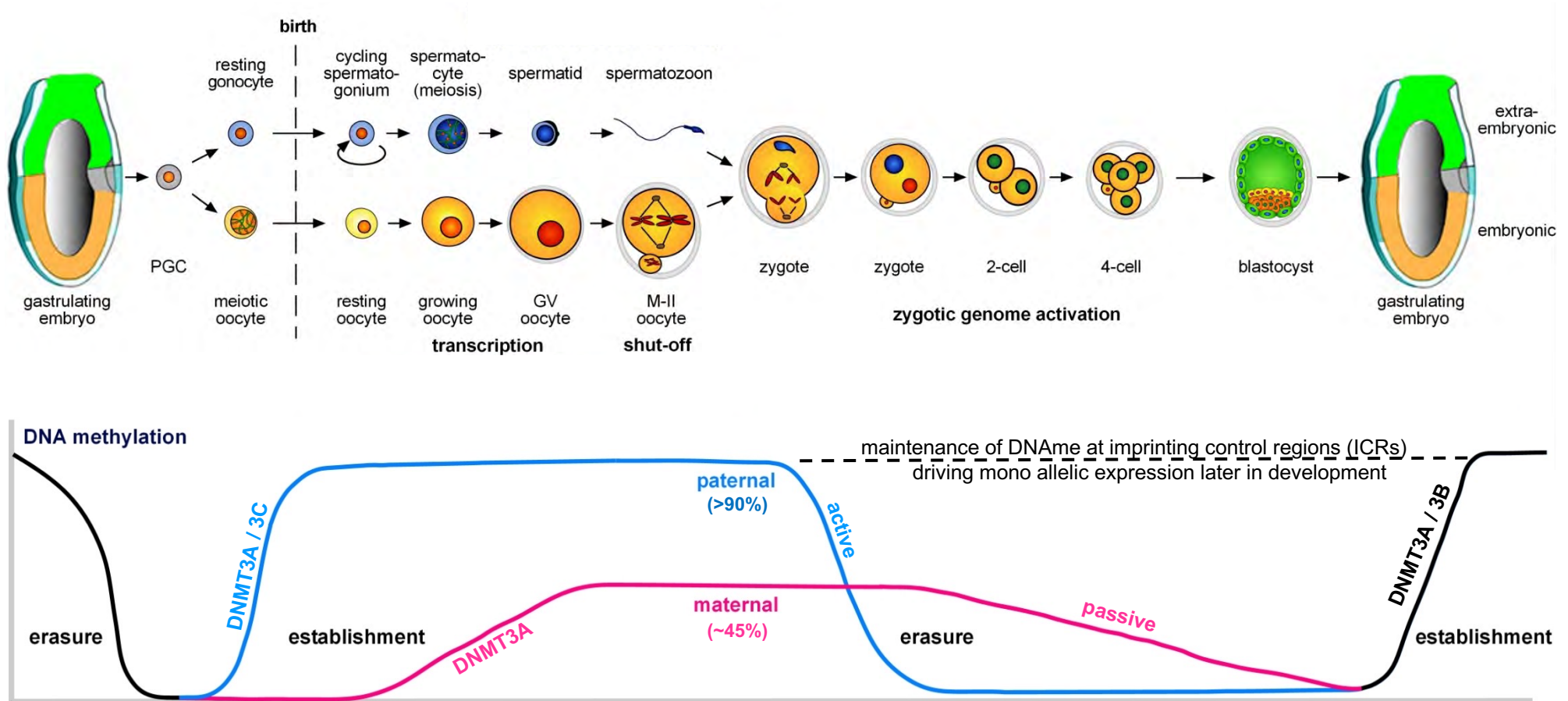
$C^{me}pG \rightarrow$ natural (chemical) deamination $\rightarrow$ improper repair $\rightarrow$ TpG

What is the mechanism underlying CpG conservation within CGIs?

or differently phrased:

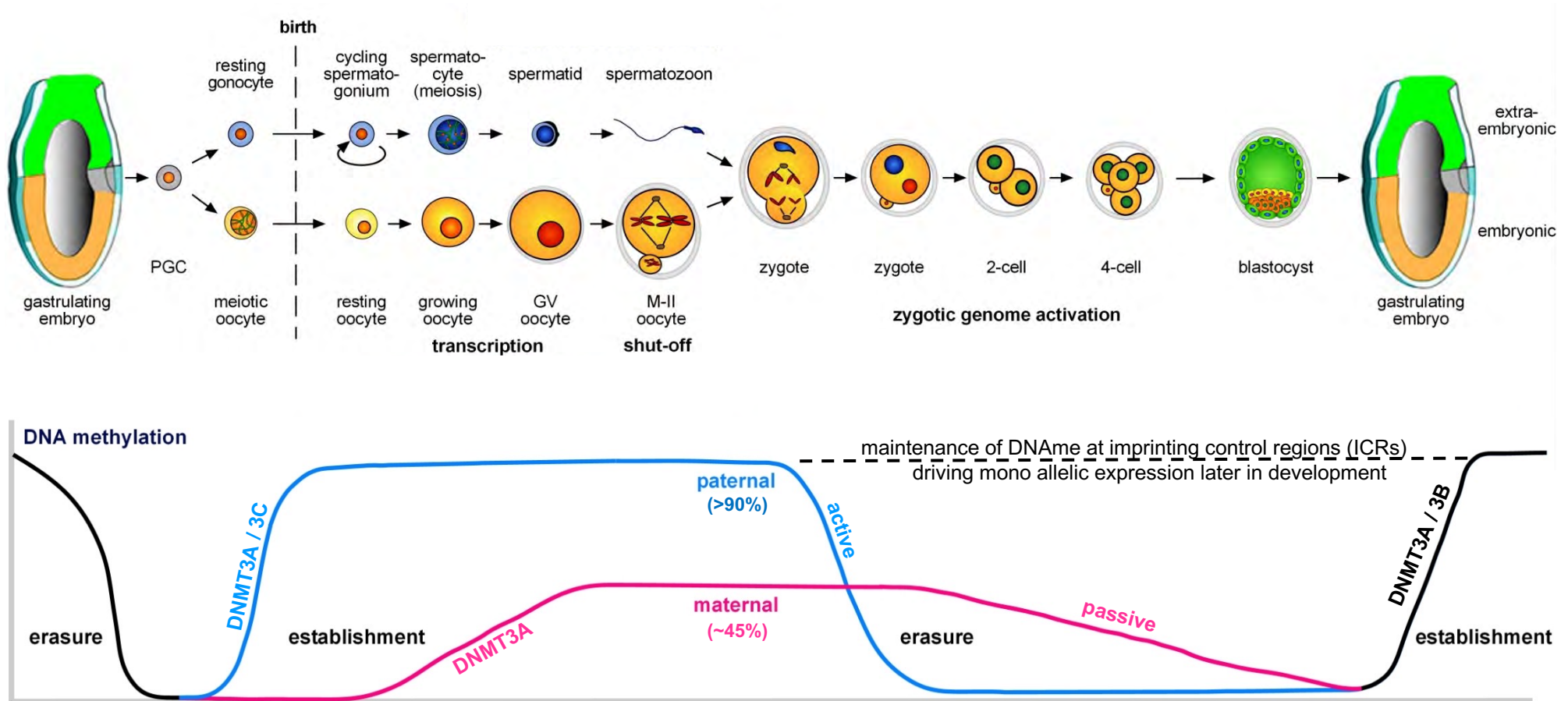
What is the mechanism preventing *de novo* DNA methylation acquisition at CGIs in the germ line?

Dynamic changes in global DNA methylation during the mammalian life cycle



Global DNA methylation levels are very different in sperm and oocytes.

Dynamic changes in global DNA methylation during the mammalian life cycle



Global DNA methylation levels are very different in sperm and oocytes.

Almost all CGIs remain unmethylated in both germ cell lineages.

→ Underlying mechanisms are unknown. Are they related?

Hyper DNA methylation at promoters of genes driving development

Gene Ontology analysis:

cell fate specification and determination, morphogenesis, cellular differentiation and cell cycle regulation

Gene families:

Transcription factors: *Foxo*, *Gata*, *Hand*, *Hes*, *Hox*, *Lhx*, *Nkx*, *Pax*, *Six*, *Sox*, *Tead*, *Tbx*, and *Zfp*.

cell signaling, growth factor activity, and cell adhesion: *Bmp*, *Dll*, *Fgf*, *Igf2*, *Pdgf*, *Pcdh*, *Rar*, *Wnt*, and *Tgfb*),

gonadal development and synaptonemal complex assembly:

Developmental processes:

blastocyst development: *Eomes*, *Gata4*, *Hnf1b*, *Junb*, *Klf4*, and *Sox17*

placenta development: *Atrx*, *Hand1*, *Lhx3*, *Vash2*, and *Wnt2*

Gastrulation: *Bmp4*, *Brachyury* [*T*], *Sox7*, and *Tlx2*