

Supplementary materials

Effects of multi-superovulation on transcription and genomic methylation of oocytes and offspring

Juan-Ke Xie¹, Qian wang¹, Yuan-Hui Chen¹, Hao-Yue Sun¹, Zhao-Jia Ge², Cui-Lian Zhang^{1*}

Figure S1 Relationship in samples.

(A) Principal component analysis (PCA) in samples; (B) sample correlation analysis.

Figure S2 Overlapped differential expression genes (DEGs) in R3 and R5 oocytes compared with R1.

Figure S3 GO analysis of overlapped DEGs.

Left panel, the GO term; right panel, enrichment of DEGs in biological process.

Figure S4 Enrichment analysis of genes in profile 0, 1, and 3.

(A-C) GO analysis of genes in profile 0, 1, and 3; (D-F) KEGG analysis of genes in profile 0, 1, and 3.

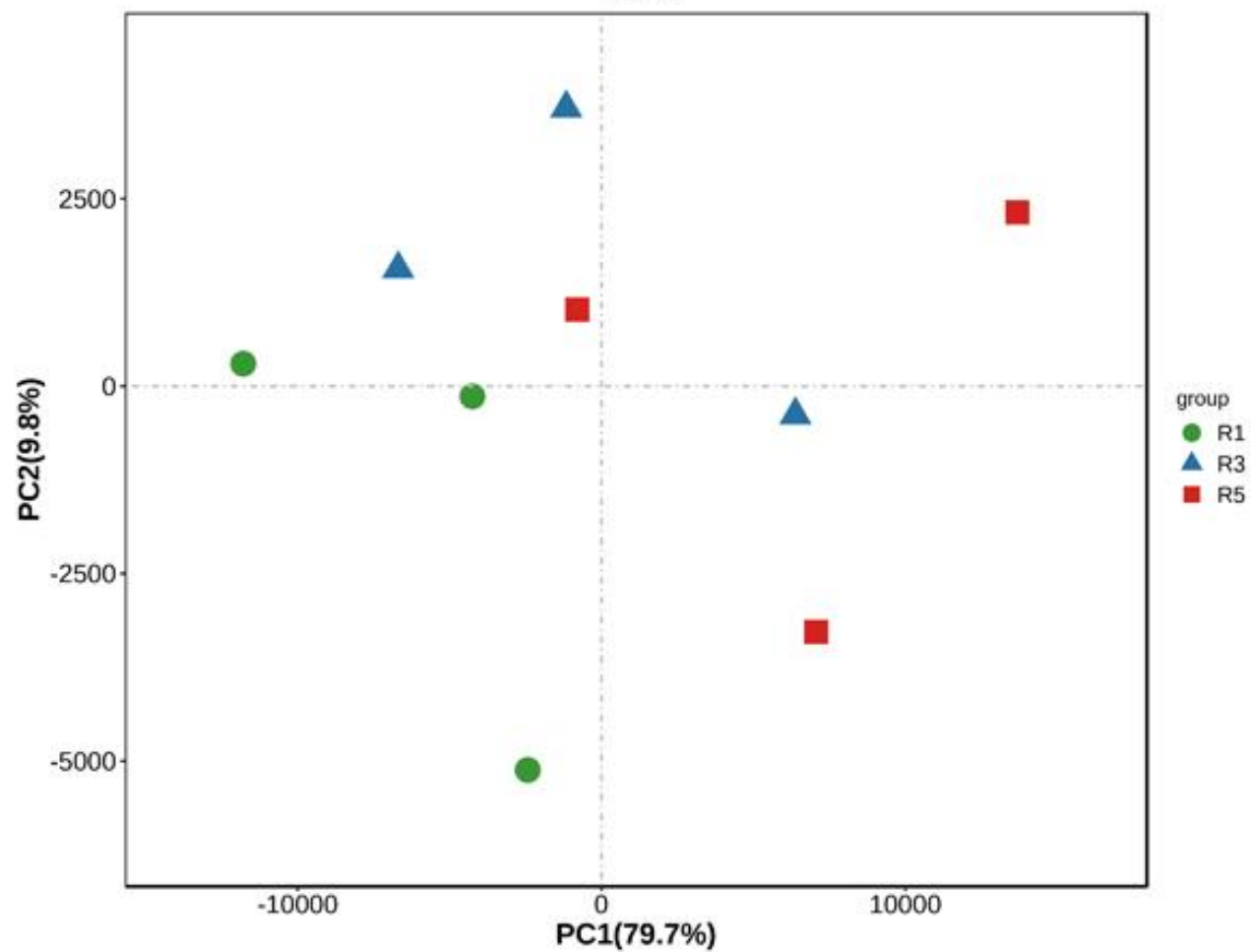
Figure S5 Distribution of hyper-DMRs in genomic elements.

Figure S6 Methylation of DMRs analyzed using bisulfite sequencing.

The methylation level of DMR1, DMR2, DMR3, and DMR4 is further analyzed using bisulfite sequencing; * $p < 0.05$, ** $p < 0.01$.

A

PCA



B

Sample Correlation

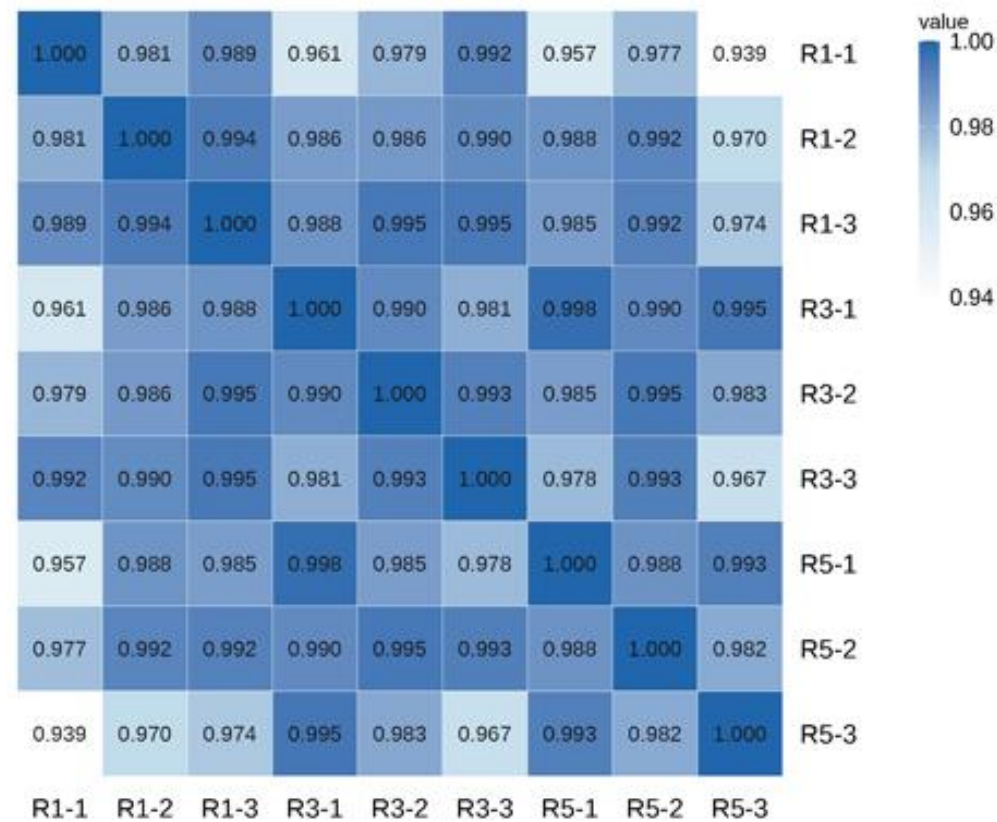


Figure S1 Relationship in samples.

(A) Principal component analysis (PCA) in samples; (B) sample correlation analysis.

DEGs

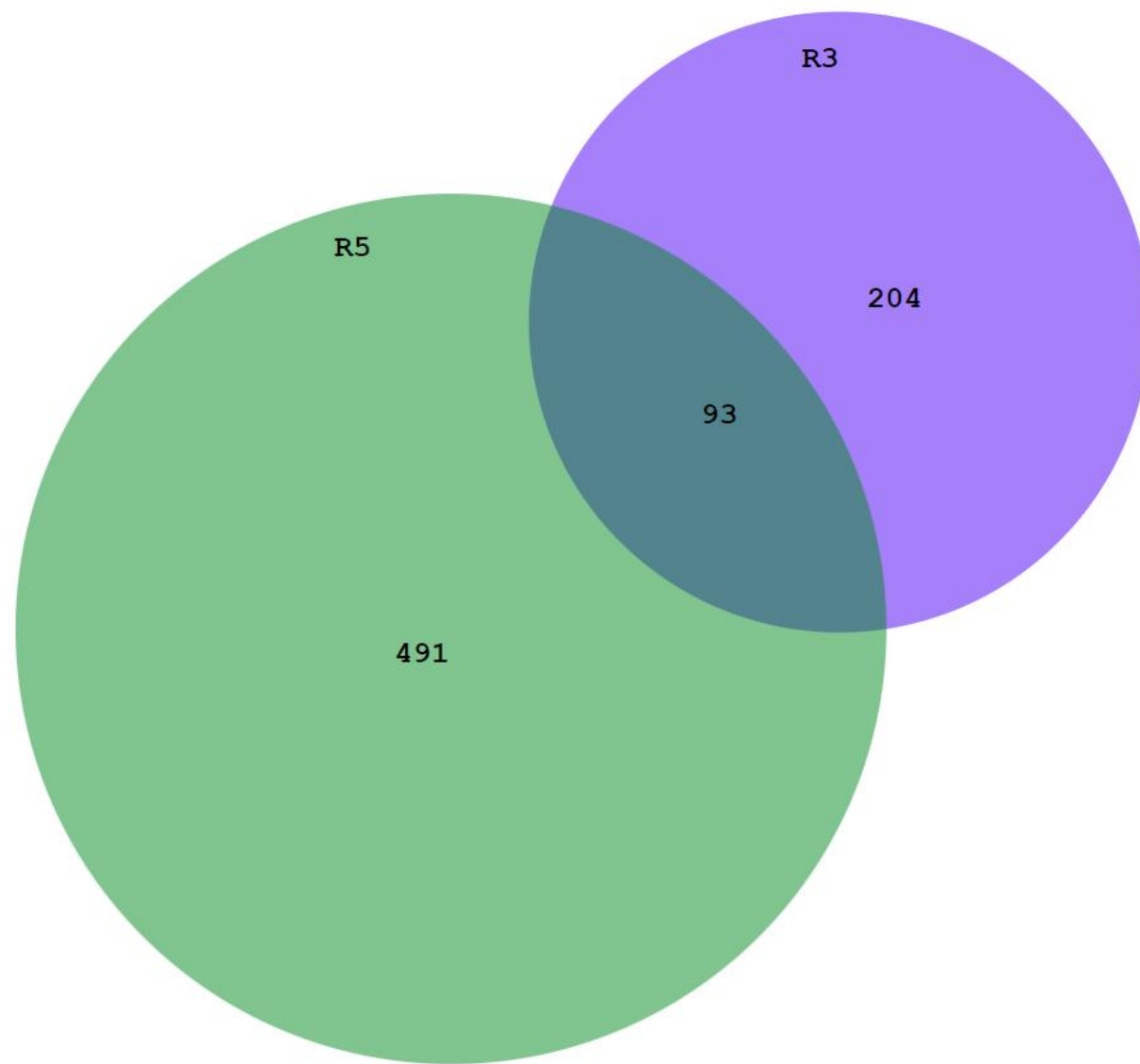


Figure S2 Overlapped differential expression genes (DEGs) in R3 and R5 oocytes compared with R1.

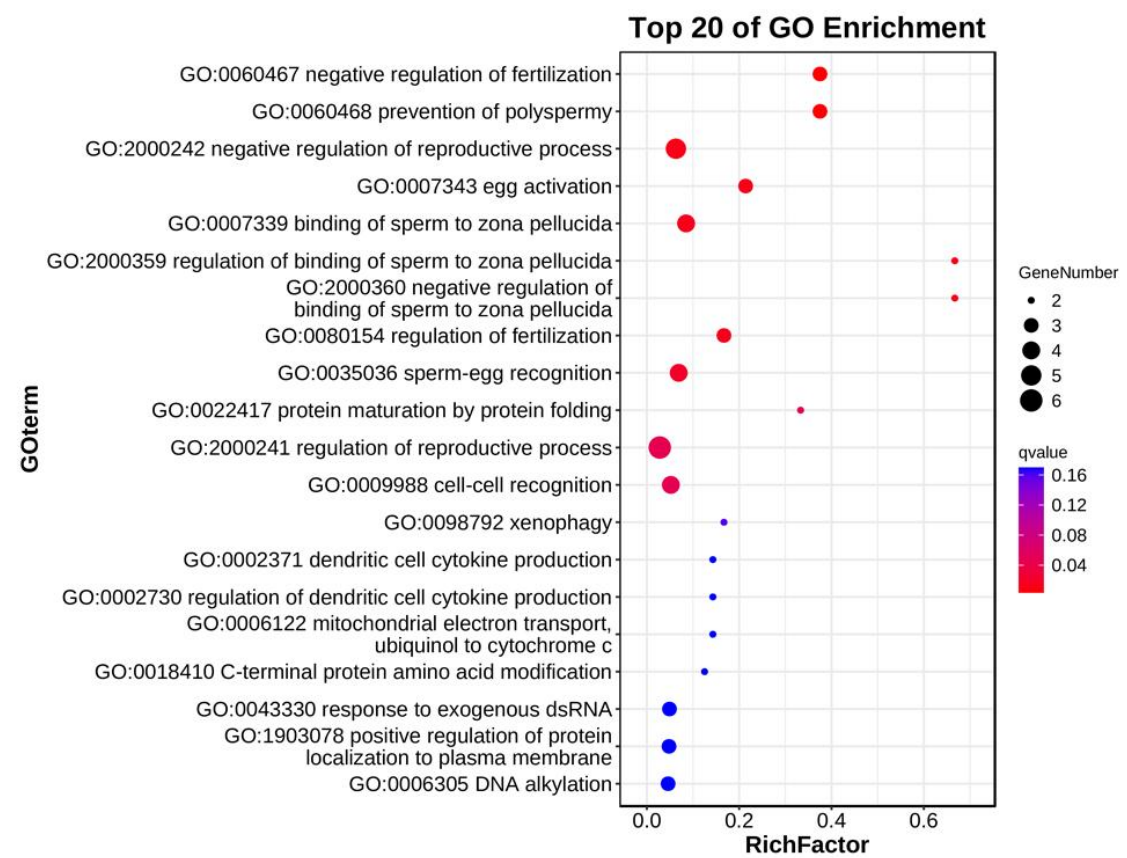
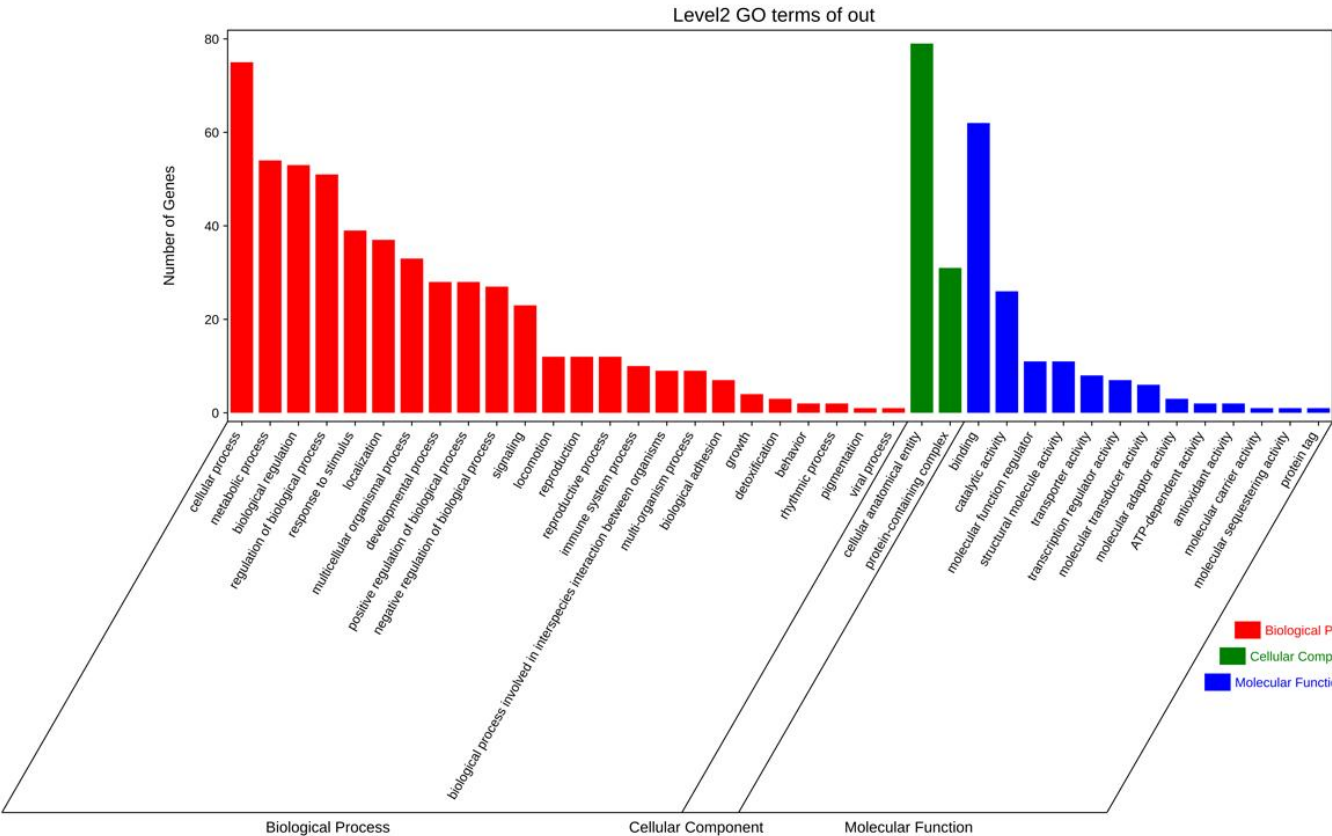


Figure S3 GO analysis of overlapped DEGs.

Left panel, the GO term; right panel, enrichment of DEGs in biological process.

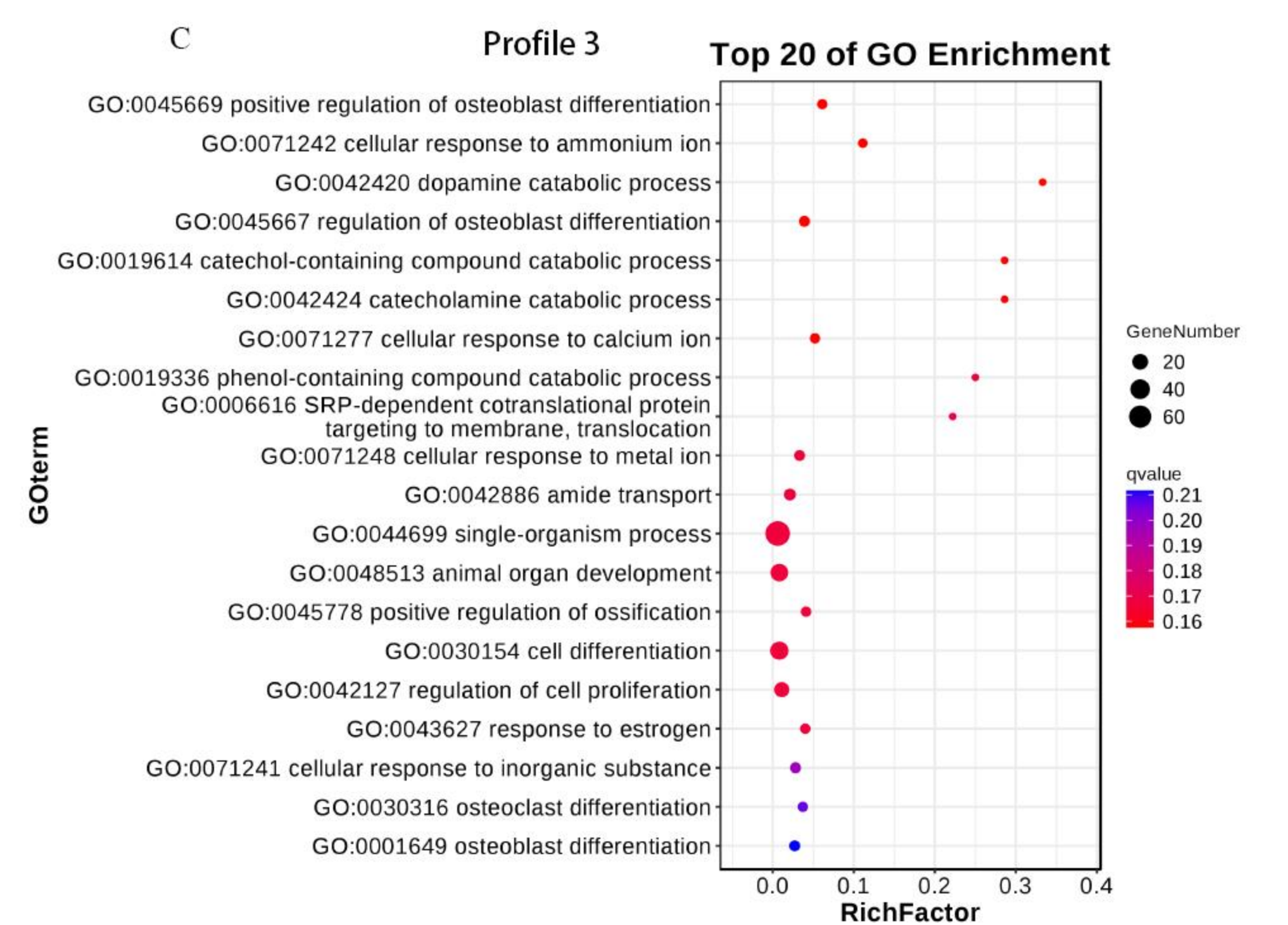
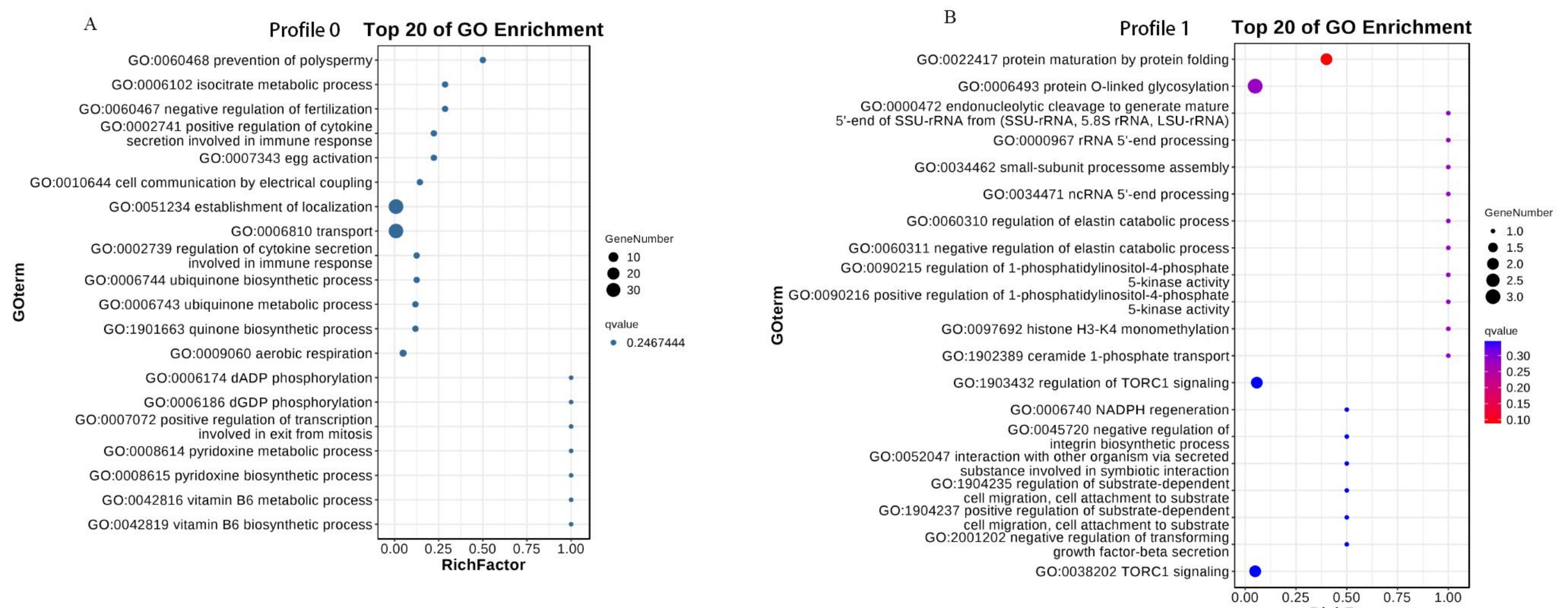
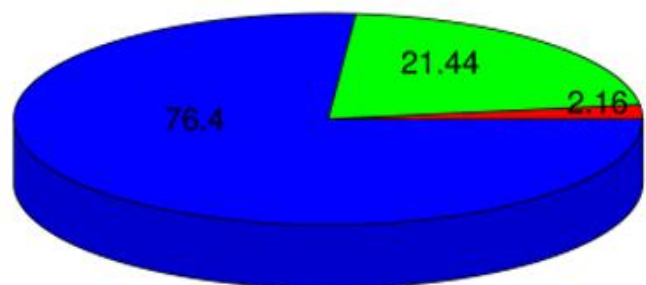
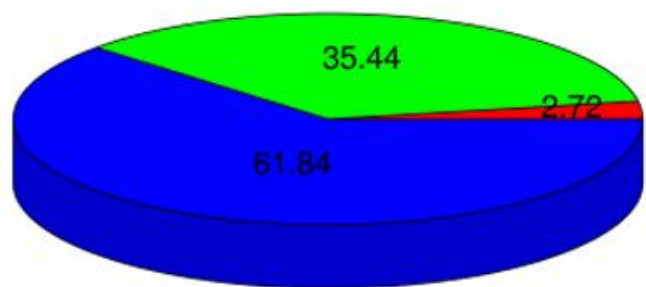
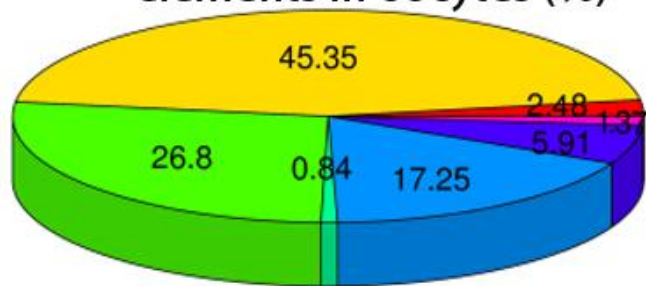


Figure S4 Enrichment analysis of genes in profile 0, 1, and 3.

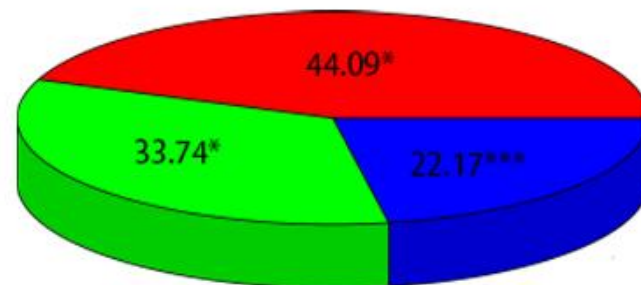
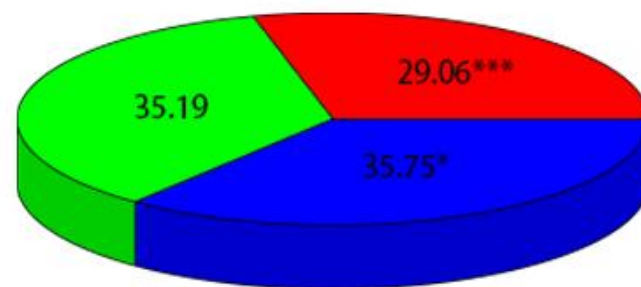
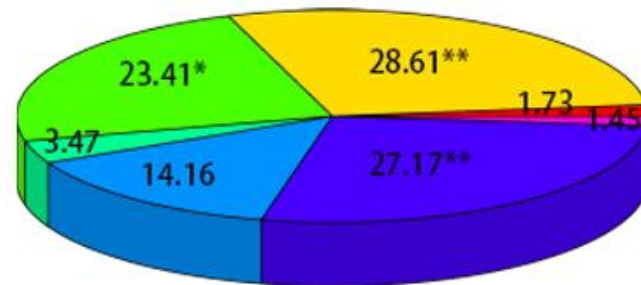
(A-C) GO analysis of genes in profile 0, 1, and 3; (D-F) KEGG analysis of genes in profile 0, 1, and 3.

Genomic distribution of elements in oocytes (%)

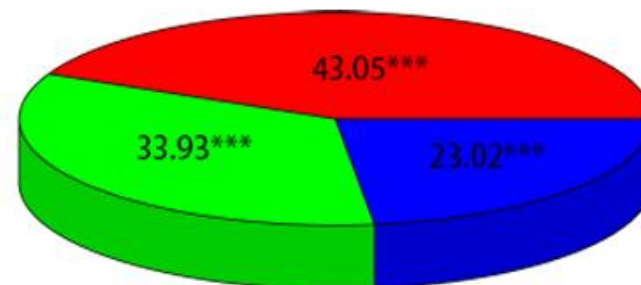
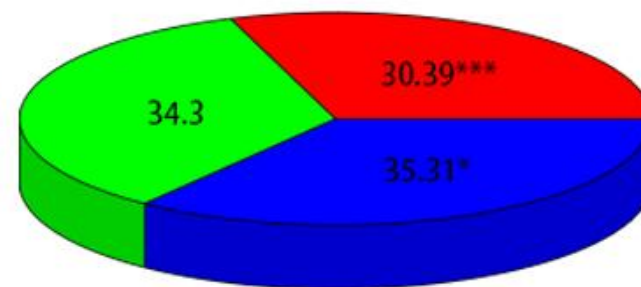
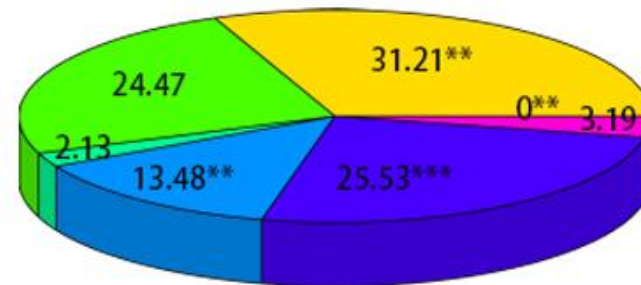


Hyper-DMRs (%)

R3 vs R1



R5 vs R1



- DNA
- LINE
- LTR
- Low_complexity
- SINE
- Simple_repeat
- Unknown

- Exon
- Intron
- Intergenic

- CGI
- CGIshore
- non.CGI

Figure S5 Distribution of hyper-DMRs in genomic elements. * $p < 0.05$, ** $p < 0.01$.

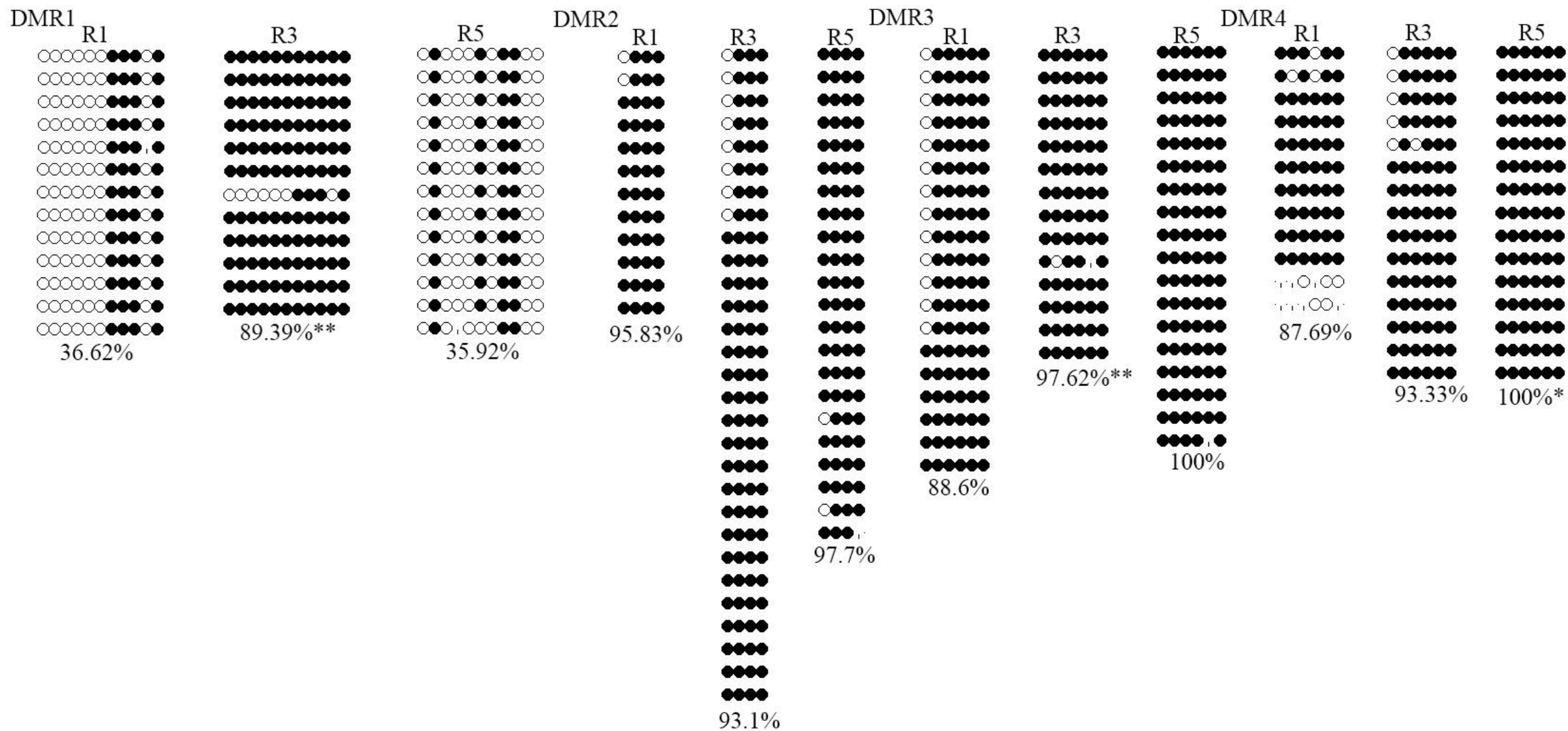


Figure S6 Methylation of DMRs analyzed using bisulfite sequencing.

The methylation level of DMR1, DMR2, DMR3, and DMR4 is further analyzed using bisulfite sequencing; * $p < 0.05$, ** $p < 0.01$.

Table S1 Primer sequences for poly (A) tail assay.

Primers	Sequence (5' to 3')
P1	GCGAGCTCCGCGGCCGCGTTTTTTTTTTTTT
padi6-P2	TTGTTGCACTGGGTGAAGG
padi6-P2-F	GGGAGTTAGGGTCTCTCACATC
pabpc11-P2	AAGCACCAGGGAGTTTGGTGAAGCTAAAGGAGTCATA
Cdk1-P2	TATTACACACCGGTCCCCTG
Eif6-P2	TTGATTGCCCAACCCTTCAC
Cops3-P2	CTGGCAAGTGTGAAGAGCG

Table S2 DEGs enriched in metabolic pathways in R3 oocytes compared with R1

Pathway	Pathway ID	K-id	Genes	K-id description
Metabolic pathways	ko01100	K00016	ncbi_16832(Ldhd)	LDH, ldh; L-lactate dehydrogenase [EC:1.1.1.27]
		K00237	ncbi_66925(Sdhd)	SDHD, SDH4; succinate dehydrogenase (ubiquinone) membrane anchor subunit
		K00323	ncbi_18115(Nnt)	NNT; H ⁺ -translocating NAD(P) transhydrogenase [EC:1.6.1.2 7.1.1.1]
		K00411	ncbi_66694(Uqcrfs1)	UQCRFS1, RIP1, petA; ubiquinol-cytochrome c reductase iron-sulfur subunit [EC:7.1.1.8]
		K00420	ncbi_66594(Uqcr11)	QCR10, UQCR; ubiquinol-cytochrome c reductase subunit 10
		K00500	ncbi_18478(Pah)	phhA, PAH; phenylalanine-4-hydroxylase [EC:1.14.16.1]
		K00605	ncbi_434437(Amt)	gcvT, AMT; aminomethyltransferase [EC:2.1.2.10]
		K00710	ncbi_108150(Galnt7)	GALNT; polypeptide N-acetylgalactosaminyltransferase [EC:2.4.1.41]
		K00710	ncbi_108148(Galnt2)	GALNT; polypeptide N-acetylgalactosaminyltransferase [EC:2.4.1.41]
		K00741	ncbi_53625(B3GNT2)	B3GNT2; N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase [EC:2.4.1.149]
		K00850	ncbi_56421(Pfkp)	pfkA, PFK; 6-phosphofructokinase 1 [EC:2.7.1.11]
		K00901	ncbi_104418(DGKZ)	dgkA, DGK; diacylglycerol kinase (ATP) [EC:2.7.1.107]
		K00939	ncbi_78801(Ak7)	adk, AK; adenylate kinase [EC:2.7.4.3]
		K00948	ncbi_110639(Prps2)	PRPS, prsA; ribose-phosphate pyrophosphokinase [EC:2.7.6.1]
		K00995	ncbi_74451(Pgs1)	pgsA, PGS1; CDP-diacylglycerol---glycerol-3-phosphate 3-phosphatidyltransferase [EC:2.7.8.5]
		K01176	ncbi_11722(Amy1)	AMY, amyA, malS; alpha-amylase [EC:3.2.1.1]
		K01578	ncbi_56690(Mlycd)	MLYCD; malonyl-CoA decarboxylase [EC:4.1.1.9]
		K01611	ncbi_11702(Amd1)	speD, AMD1; S-adenosylmethionine decarboxylase [EC:4.1.1.50]
		K03882	ncbi_17720(Mtnd4l)	ND4L; NADH-ubiquinone oxidoreductase chain 4L [EC:7.1.1.2]
		K03949	ncbi_68202(Ndufa5)	NDUFA5; NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 5

K05605	ncbi_227095(Hibch)	HIBCH; 3-hydroxyisobutyryl-CoA hydrolase [EC:3.1.2.4]
K06210	ncbi_66454(Nmnat1)	NMNAT; nicotinamide mononucleotide adenylyltransferase [EC:2.7.7.1 2.7.7.18]
K12235	ncbi_27364(Srr)	SRR; serine racemase [EC:5.1.1.18]
K12670	ncbi_13200(Ddost)	WBP1; oligosaccharyltransferase complex subunit beta
K13485	ncbi_231903(Urad)	PRHOXNB, URAD; 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase [EC:4.1.1.97]
K14085	ncbi_110695(Aldh7a1)	ALDH7A1; aldehyde dehydrogenase family 7 member A1 [EC:1.2.1.31 1.2.1.8 1.2.1.3]
K16816	ncbi_66853(Pnpla2)	PNPLA2, ATGL; patatin-like phospholipase domain-containing protein 2 [EC:3.1.1.3]
K18086	ncbi_97287(Mtmt14)	MTMR14; myotubularin-related protein 14 [EC:3.1.3.64 3.1.3.95]

Table S3 DEGs enriched in metabolic pathways in R5 oocytes compared with R1

Pathway	Pathway ID	K-id	Genes	K-id description
Metabolic pathway	K01100	K00016	ncbi_16832(Ldhb)	LDH, ldh; L-lactate dehydrogenase [EC:1.1.1.27]
		K00030	ncbi_170718(Idh3B)	IDH3; isocitrate dehydrogenase (NAD+) [EC:1.1.1.41]
		K00254	ncbi_56749(Dhodh)	DHODH, pyrD; dihydroorotate dehydrogenase [EC:1.3.5.2]
		K00275	ncbi_103711(Pnpo)	pdxH, PNPO; pyridoxamine 5'-phosphate oxidase [EC:1.4.3.5]
		K00420	ncbi_66594(Uqcr11)	QCR10, UQCR; ubiquinol-cytochrome c reductase subunit 10
		K00489	ncbi_13122(Cyp7a1)	CYP7A1; cholesterol 7alpha-monooxygenase [EC:1.14.14.23]
		K00591	ncbi_230027(Coq3)	COQ3; polyprenyldihydroxybenzoate methyltransferase / 3-demethylubiquinol 3-O-methyltransferase [EC:2.1.1.114 2.1.1.64]
				GBE1, glgB; 1,4-alpha-glucan branching enzyme [EC:2.4.1.18]
		K00700	ncbi_74185(Gbe1)	GALNT; polypeptide
		K00710	ncbi_108150(Galnt7)	N-acetylgalactosaminyltransferase [EC:2.4.1.41]
		K00719	ncbi_26879(B3galnt1)	B3GALNT1, B3GALT3; galactosylgalactosylglucosylceramide
		K00733	ncbi_218271(B4galt7)	beta-D-acetylgalactosaminyltransferase [EC:2.4.1.79]
				B4GALT7; xylosylprotein 4-beta-galactosyltransferase [EC:2.4.1.133]
		K00737	ncbi_17309(Mgat3)	MGAT3; beta-1,4-mannosyl-glycoprotein beta-1,4-N-acetylglucosaminyltransferase [EC:2.4.1.144]

K00741	ncbi_53625(B3GNT2)	B3GNT2; N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase [EC:2.4.1.149]
K00789	ncbi_232087(Mat2a)	metK; S-adenosylmethionine synthetase [EC:2.5.1.6]
K01068	ncbi_171210(Acot2)	ACOT1_2_4; acyl-coenzyme A thioesterase 1/2/4 [EC:3.1.2.2]
K01092	ncbi_55980(Impa1)	E3.1.3.25, IMPA, suhB; myo-inositol-1(or 4)-monophosphatase [EC:3.1.3.25]
K01196	ncbi_77559(AGL)	AGL; glycogen debranching enzyme [EC:2.4.1.25 3.2.1.33]
K01581	ncbi_18263(Odc1)	E4.1.1.17, ODC1, speC, speF; ornithine decarboxylase [EC:4.1.1.17]
K01611	ncbi_100041585(Amd2)	speD, AMD1; S-adenosylmethionine decarboxylase [EC:4.1.1.50]
K01681	ncbi_11429(Aco2)	ACO, acnA; aconitate hydratase [EC:4.2.1.3]
K01897	ncbi_74205(Acsl3)	ACSL, fadD; long-chain acyl-CoA synthetase [EC:6.2.1.3]
K01908	ncbi_380660(Acss3)	ACSS3, prpE; propionyl-CoA synthetase [EC:6.2.1.17]
K02129	ncbi_11958(Atp5me)	ATPeF0E, ATP5I; F-type H ⁺ -transporting ATPase subunit e
K02130	ncbi_57423(Atp5mf)	ATPeF0F, ATP5J2; F-type H ⁺ -transporting ATPase subunit f
K02131	ncbi_11957(Atp5pf)	ATPeF0F6, ATP5J; F-type H ⁺ -transporting ATPase subunit 6
K02147	ncbi_11966(Atp6v1b2)	ATPeV1B, ATP6B; V-type H ⁺ -transporting ATPase subunit B
K02266	ncbi_12861(Cox6a1)	COX6A; cytochrome c oxidase subunit 6a
K02321	ncbi_18969(Pola2)	POLA2; DNA polymerase alpha subunit B

K02327	ncbi_18971(Pold1)	POLD1; DNA polymerase delta subunit 1 [EC:2.7.7.7]
K02564	ncbi_26384(Gnpda1)	nagB, GNPDA; glucosamine-6-phosphate deaminase [EC:3.5.99.6]
K03017	ncbi_69920(POLR2I)	RPB9, POLR2I; DNA-directed RNA polymerase II subunit RPB9
K03662	ncbi_54411(Atp6ap1)	ATPeVS1, ATP6S1; V-type H ⁺ -transporting ATPase S1 subunit
K03843	ncbi_56737(Alg2)	ALG2; alpha-1,3/alpha-1,6-mannosyltransferase [EC:2.4.1.132 2.4.1.257]
K03847	ncbi_223774(Alg12)	ALG12; alpha-1,6-mannosyltransferase [EC:2.4.1.260]
K03882	ncbi_17720(Mtnd4l))	ND4L; NADH-ubiquinone oxidoreductase chain 4L [EC:7.1.1.2]
K03939	ncbi_407785(Ndufs6)	NDUFS6; NADH dehydrogenase (ubiquinone) Fe-S protein 6
K04710	ncbi_76893(Cers2)	CERS1_2_3_4, LASS1_2_3_4; ceramide synthetase [EC:2.3.1.24]
K05285	ncbi_27392(Pign)	PIGN; GPI ethanolamine phosphate transferase 1 [EC:2.7.-.-]
K06127	ncbi_52064(Coq5)	COQ5; 2-methoxy-6-polyprenyl-1,4-benzoquinol methylase [EC:2.1.1.201]
K06210	ncbi_66454(Nmnat1)	NMNAT; nicotinamide mononucleotide adenylyltransferase [EC:2.7.7.1 2.7.7.18]
K07413	ncbi_226143(Cyp2c23)	CYP2C; cytochrome P450 family 2 subfamily C [EC:1.14.14.1]

K07512	ncbi_26922(Mecr)	MECR, NRBF1; mitochondrial enoyl-[acyl-carrier protein] reductase / trans-2-enoyl-CoA reductase [EC:1.3.1.- 1.3.1.38]
K12670	ncbi_13200(Ddost)	WBP1; oligosaccharyltransferase complex subunit beta
K13510	ncbi_210992(Lpcat1)	LPCAT1_2; lysophosphatidylcholine acyltransferase / lyso-PAF acetyltransferase [EC:2.3.1.23 2.3.1.67]
K13534	ncbi_116939(Pnpla3)	PNPLA3; patatin-like phospholipase domain-containing protein 3 [EC:3.1.1.3 2.3.1.-]
K14085	ncbi_110695(Aldh7a1)	ALDH7A1; aldehyde dehydrogenase family 7 member A1 [EC:1.2.1.31 1.2.1.8 1.2.1.3]
K15730	ncbi_56351(Ptges3)	PTGES3; cytosolic prostaglandin-E synthase [EC:5.3.99.3]
K18086	ncbi_97287(Mtmr14)	MTMR14; myotubularin-related protein 14 [EC:3.1.3.64 3.1.3.95]
K18533	ncbi_633979(AK9)	AK9; adenylate/nucleoside-diphosphat e kinase [EC:2.7.4.3 2.7.4.6]
