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Supplemental information

**Developmental alterations in DNA
methylation during gametogenesis
from primordial germ cells to sperm**

Millissia Ben Maamar, Daniel Beck, Eric Nilsson, John R. McCarrey, and Michael K. Skinner

Developmental Alterations in DNA Methylation During Gametogenesis from Primordial Germ Cells to Sperm

Millissia Ben Maamar¹, Daniel Beck¹, Eric Nilsson¹, John R. McCarrey² and
*Michael K. Skinner¹

SUPPLEMENTAL MATERIAL

Supplemental Figure and Table Legends

Supplemental Figure S1. The number of DMRs at different CpG densities. All DMRs at a p-value threshold for top 1000 DMR are presented. The number of DMR at different CpG sites per 100 bp presented. **(A)** E13 primordial germ cells versus E16 prospermatogonia. **(B)** E16 prospermatogonia versus P10 spermatogonia. **(C)** P10 spermatogonia versus pachytene spermatocytes. **(D)** Pachytene spermatocytes versus round spermatids. **(E)** Round spermatids versus caput epididymal spermatozoa. **(F)** Caput epididymal spermatozoa versus cauda sperm.

Supplemental Figure S2. The number of DMRs at different CpG densities. All DMRs at a p-value threshold of $1e-05$ are presented. **(A)** E13 primordial germ cells versus E16 prospermatogonia. **(B)** E16 prospermatogonia versus P10 spermatogonia. **(C)** P10 spermatogonia versus pachytene spermatocytes. **(D)** Pachytene spermatocytes versus round spermatids. **(E)** Round spermatids versus caput epididymal spermatozoa. **(F)** Caput epididymal spermatozoa versus cauda sperm.

Supplemental Figure S3. The number of DMR at different lengths (kilobases). All DMRs at a p-value threshold for top 1000 DMR are presented. The number of DMR at different length (kilobase) presented. **(A)** E13 primordial germ cells versus E16 prospermatogonia. **(B)** E16 prospermatogonia versus P10 spermatogonia. **(C)** P10 spermatogonia versus pachytene spermatocytes. **(D)** Pachytene spermatocytes versus

round spermatids. **(E)** Round spermatids versus caput epididymal spermatozoa. **(F)** Caput epididymal spermatozoa versus cauda sperm.

Supplemental Figure S4. The number of DMR at different lengths. All DMRs at a p-value threshold of $1e-05$ are presented. **(A)** E13 primordial germ cells versus E16 prospermatogonia. **(B)** E16 prospermatogonia versus P10 spermatogonia. **(C)** P10 spermatogonia versus pachytene spermatocytes. **(D)** Pachytene spermatocytes versus round spermatids. **(E)** Round spermatids versus caput epididymal spermatozoa. **(F)** Caput epididymal spermatozoa versus cauda sperm.

Supplemental Figure S5. Principal component analysis (PCA) of DMR genome-wide RPKM read depth at $p < 1e-05$. **(A)** E13 primordial germ cells versus E16 prospermatogonia. **(B)** E16 prospermatogonia versus P10 spermatogonia. **(C)** P10 spermatogonia versus pachytene spermatocytes. **(D)** Pachytene spermatocytes versus round spermatids. **(E)** Round spermatids versus caput epididymal spermatozoa. **(F)** Caput epididymal spermatozoa versus cauda sperm.

Supplemental Figure S6. Top 100 developmental stage methylation sites read depth (RPKM) comparisons. **(A)** Caput spermatozoa top 100 sites, **(B)** Round spermatid top 100 sites, **(C)** Pachytene spermatocyte top 100 sites, **(D)** Spermatozoa top 100 sites, **(E)** Caput spermatozoa top 100 sites, and **(F)** Primordial germ cell top 100 sites.

Supplemental Figure S7. DMR associated gene pathways. KEGG gene pathways: PGC versus E16 prospermatogonia, prospermatogonia versus spermatogonia, spermatogonia versus pachytene, pachytene versus round spermatids, round spermatids versus caput, caput versus cauda. The KEGG pathway number, name and number of associated genes in brackets.

Supplemental Figure S8. WGCNA dendrogram for the different modules. The dendrogram height versus colored module is presented for all the modules. This figure shows how the genomic windows cluster into modules. The genomic windows are split into manageable sub-groups prior to clustering to keep computational requirements low.

Supplemental Figure S9. WGCNA module DNA methylation site chromosomal locations. Each module DNA methylation sites are presented on the chromosomal plot as a red arrowhead. The chromosome number and size of the chromosome (Mb) is presented. Modules are (A) black, (B) cyan, (C) green yellow, (D) grey, (E) grey 60, (F) light cyan, (G) magenta, (H) midnight blue, (I) pink, (J) purple, (K) red, (L) salmon, (M) tan. Blue, brown and turquoise not presented due to high number of methylation sites.

Supplemental Figure S10. WGCNA module DNA methylation site overlap with DMRs at various developmental stage comparisons. The color modules compared to specific developmental stage comparisons for DMR overlap with module DNA methylation sites are presented.

Supplemental Table S1. DMR site list PGC versus prospermatogonia top 1000 DMR.

DMR name, chromosome, start, stop, length, number signature windows, minimum p-value, max log-fold change, CpG number, CpG density, gene annotation, and gene category are presented.

Supplemental Table S2. DMR site list prospermatogonia versus spermatogonia top

1000 DMR. DMR name, chromosome, start, stop, length, number signature windows, minimum p-value, max log-fold change, CpG number, CpG density, gene annotation, and gene category are presented.

Supplemental Table S3. DMR site list spermatogonia versus pachytene top 1000

DMR. DMR name, chromosome, start, stop, length, number signature windows, minimum p-value, max log-fold change, CpG number, CpG density, gene annotation, and gene category are presented.

Supplemental Table S4. DMR site list pachytene versus round spermatid top 1000

DMR. DMR name, chromosome, start, stop, length, number signature windows, minimum p-value, max log-fold change, CpG number, CpG density, gene annotation, and gene category are presented.

Supplemental Table S5. DMR site list round spermatid versus caput spermatozoa

top 1000 DMR. DMR name, chromosome, start, stop, length, number signature windows,

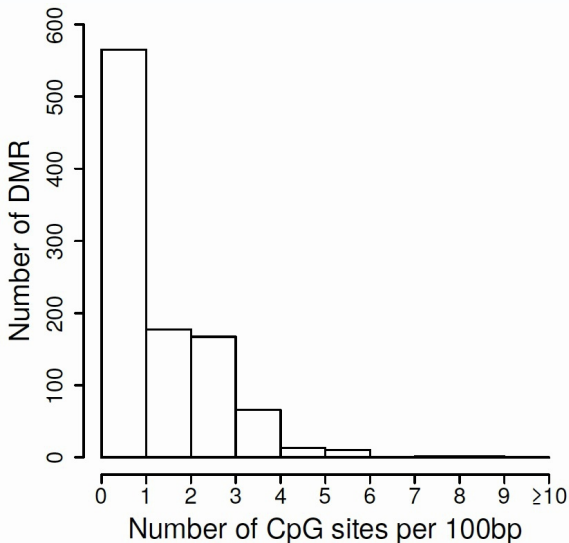
minimum p-value, max log-fold change, CpG number, CpG density, gene annotation, and gene category are presented.

Supplemental Table S6. DMR site list caput versus cauda top 1000 DMR. DMR name, chromosome, start, stop, length, number signature windows, minimum p-value, max log-fold change, CpG number, CpG density, gene annotation, and gene category are presented.

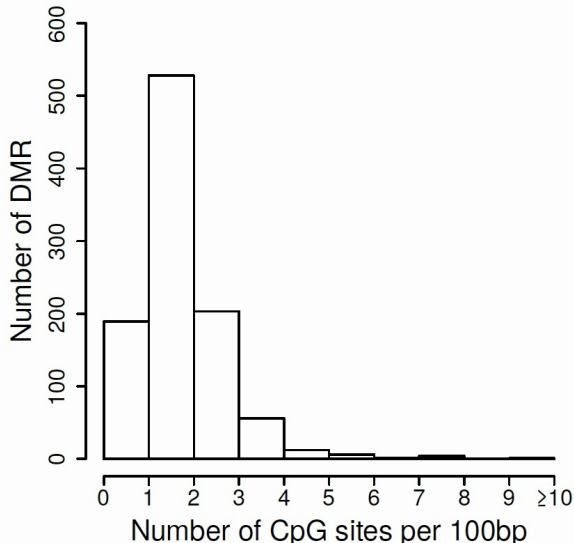
Supplemental Table S7. DMR overlap with Red module DNA methylation sites for prospermatogonia stage. The 451 DMRs overlapped were used to identify DMR associated genes, Supplemental Figure S7, and used for a network analysis. DMR name, chromosomal site, start and stop sites, gene annotation and gene category are presented.

Supplemental Figure S1

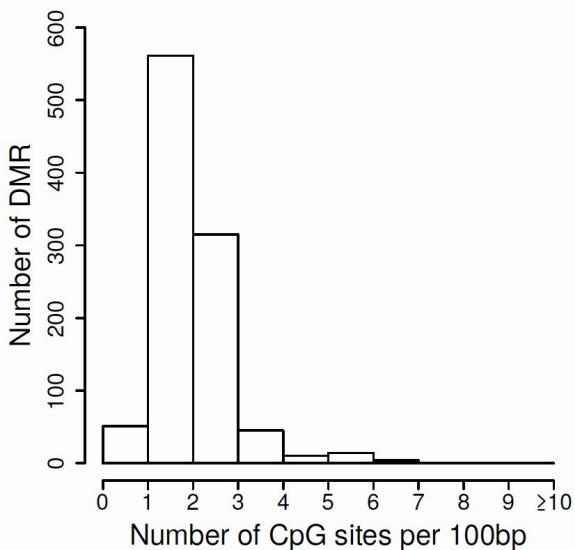
A E13 Primordial Germ Cells vs E16 Prospermatogonia



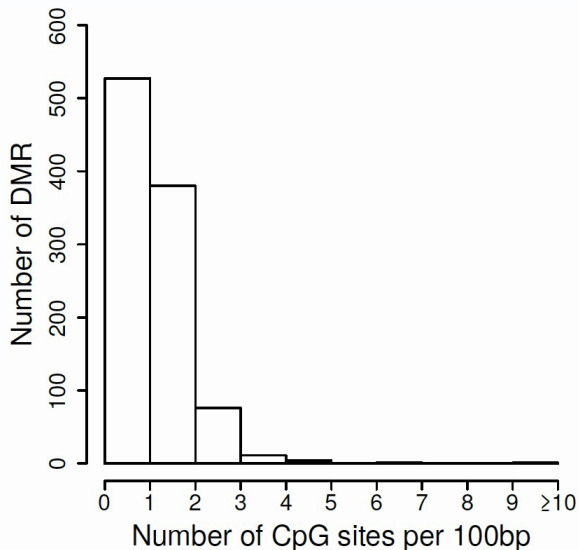
B E16 Prospermatogonia vs P10 Spermatogonia



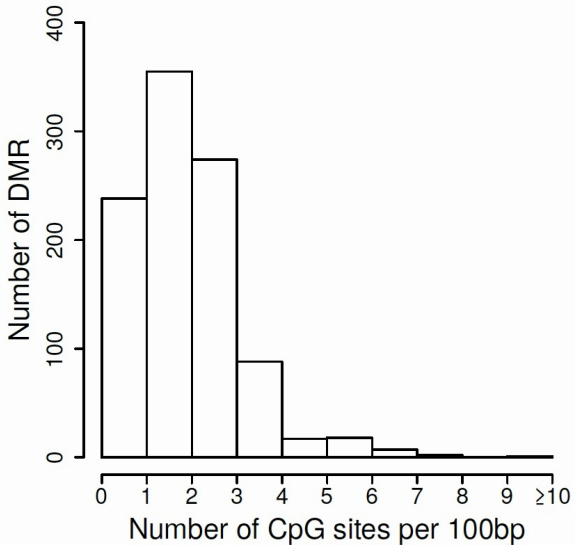
C P10 Spermatogonia vs Pachytene Spermatocytes



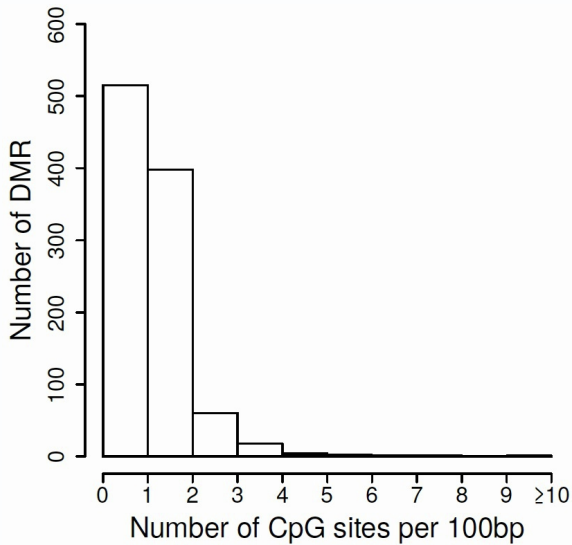
D Pachytene Spermatocytes vs Round Spermatids



E Round Spermatids vs Caput Epididymal Spermatozoa

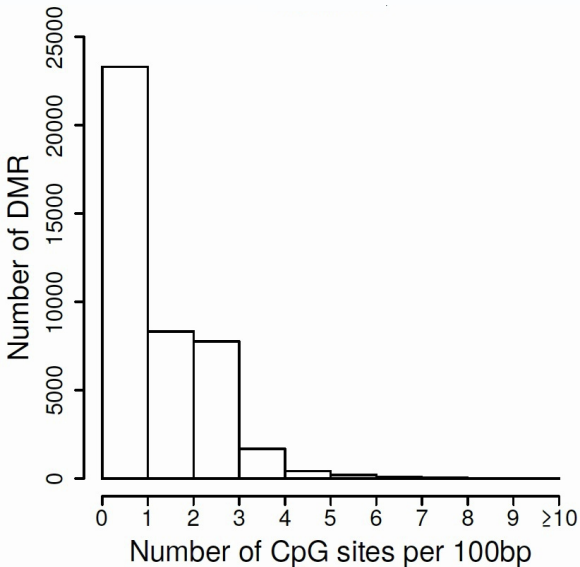


F Caput Epididymal Spermatozoa vs Cauda Sperm

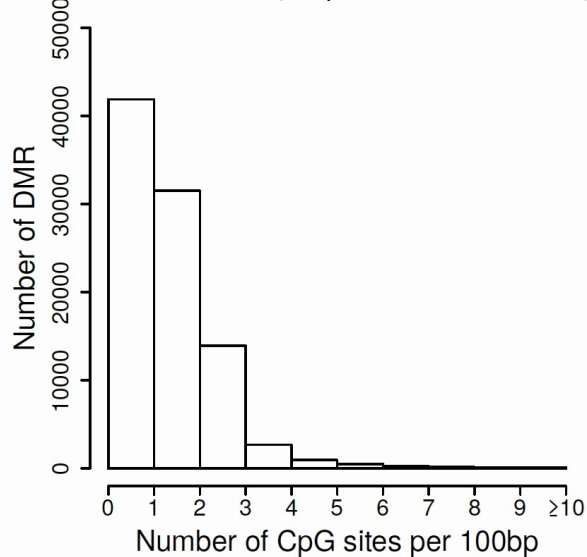


Supplemental Figure S2

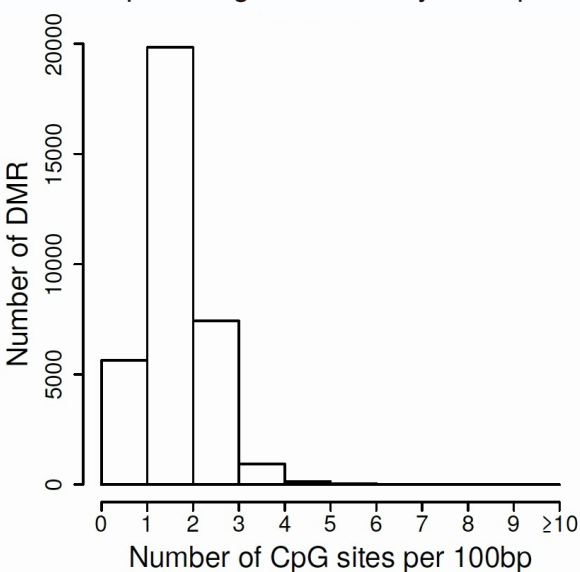
A E13 Primordial Germ Cells vs E16 Prospermatogonia



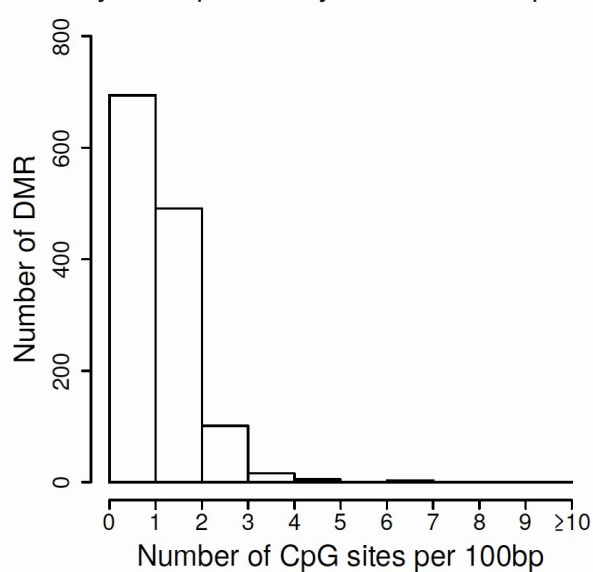
B E16 Prospermatogonia vs P10 Spermatogonia



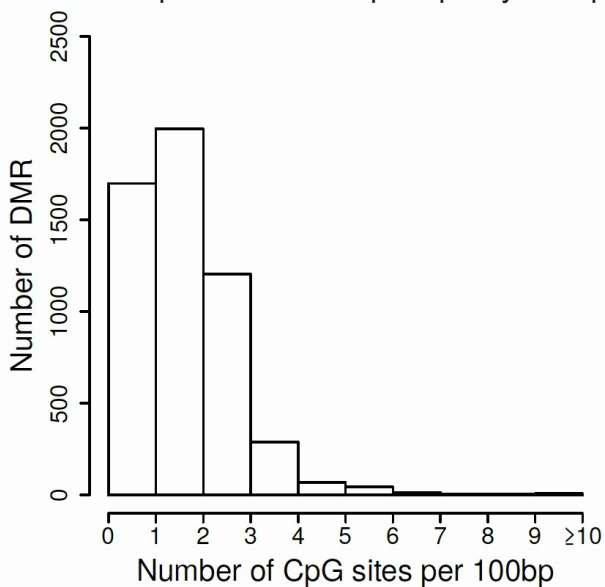
C P10 Spermatogonia vs Pachytene Spermatocytes



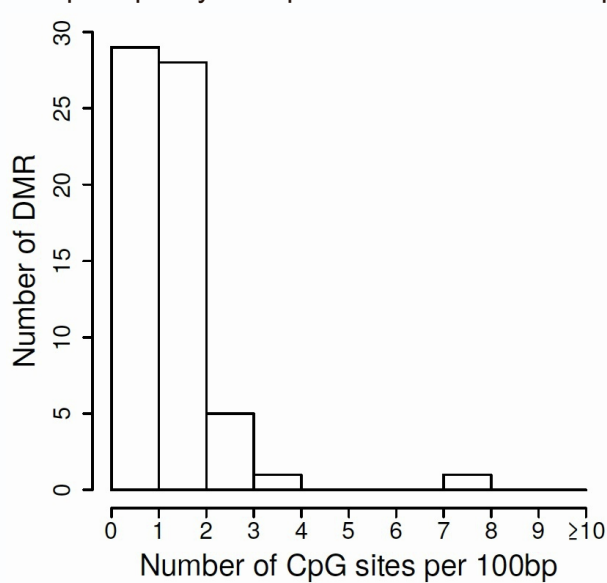
D Pachytene Spermatocytes vs Round Spermatids



E Round Spermatids vs Caput Epididymal Spermatozoa

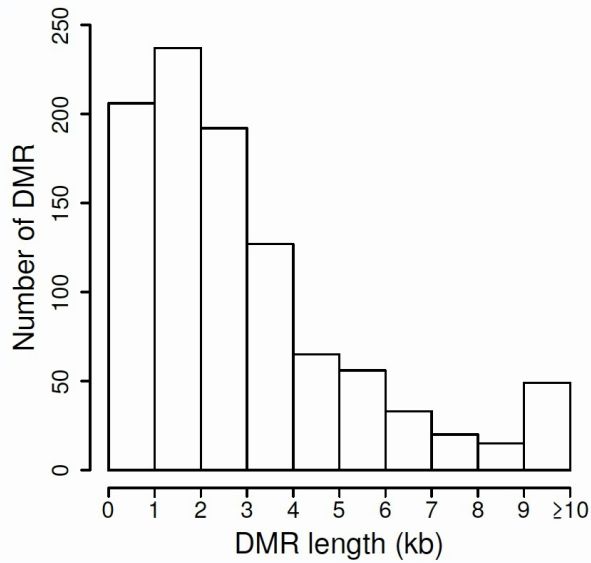


F Caput Epididymal Spermatozoa vs Cauda Spermatozoa

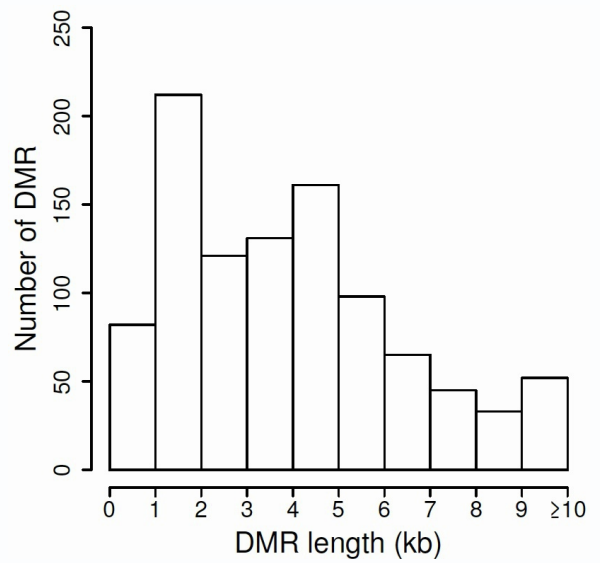


Supplemental Figure S3

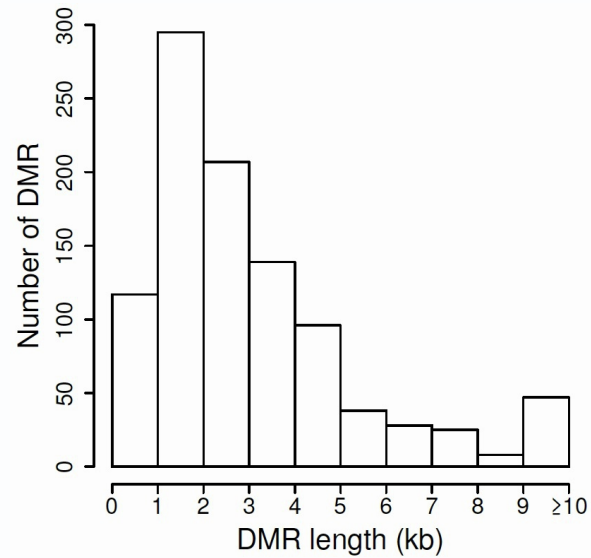
A E13 Primordial Germ Cells vs E16 Prospermatogonia



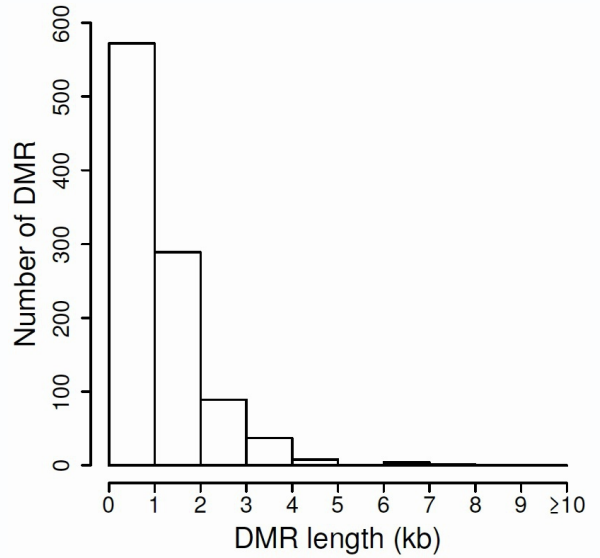
B E16 Prospermatogonia vs P10 Spermatogonia



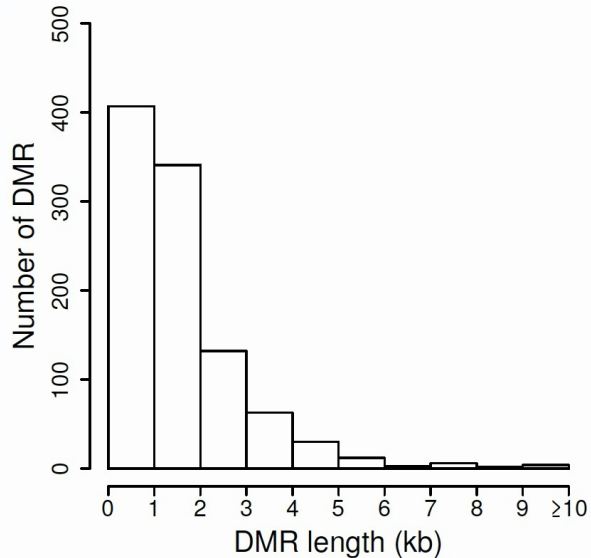
C P10 Spermatogonia vs Pachytene Spermatocytes



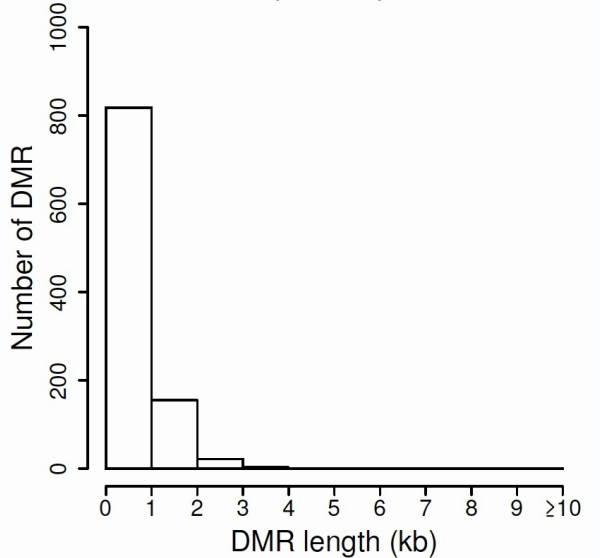
D Pachytene Spermatocytes vs Round Spermatids



E Round Spermatids vs Caput Epididymal Spermatozoa

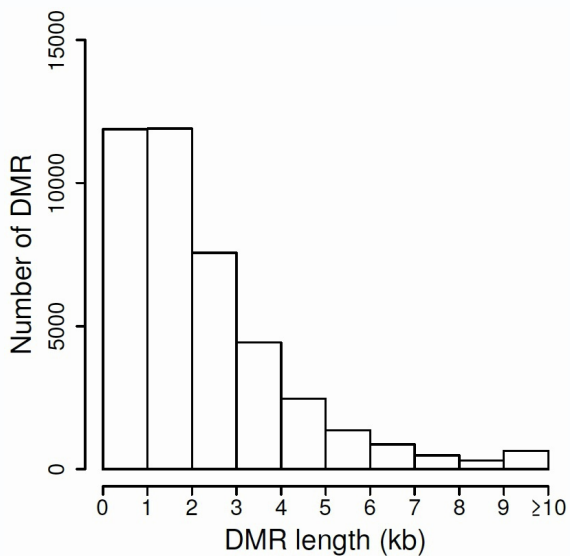


F Caput Epididymal Spermatozoa vs Cauda Spermatozoa

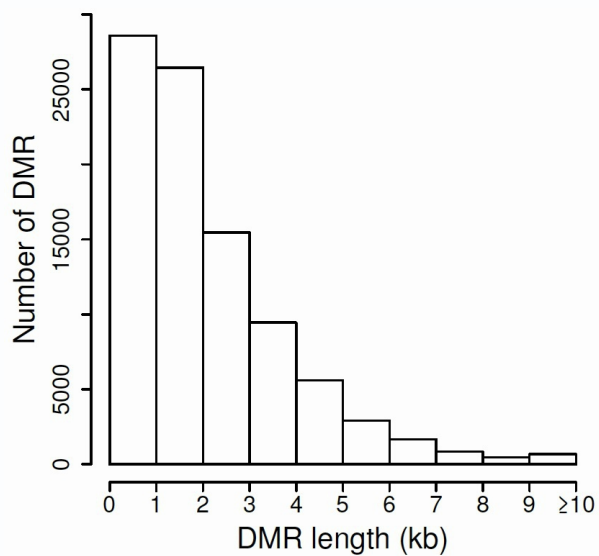


Supplemental Figure S4

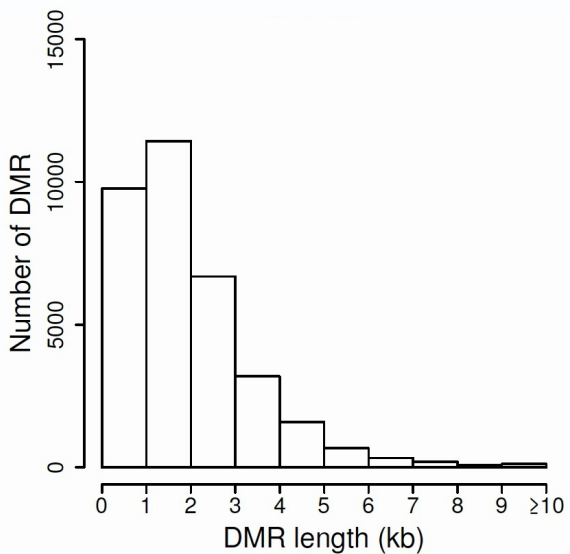
A E13 Primordial Germ Cells vs E16 Prospermatogonia



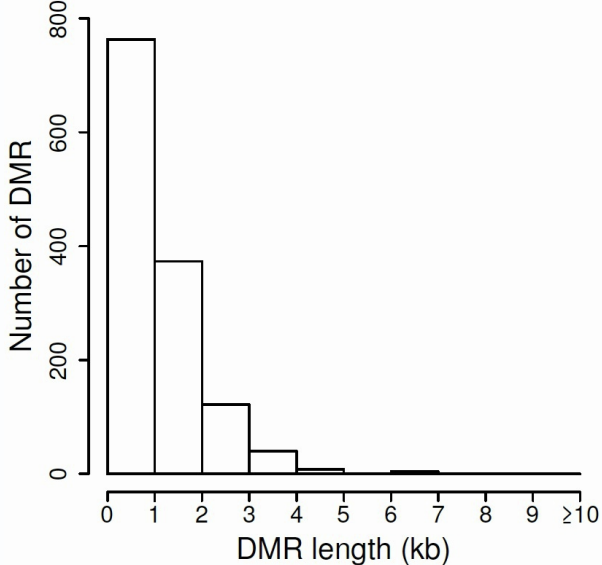
B E16 Prospermatogonia vs P10 Spermatogonia



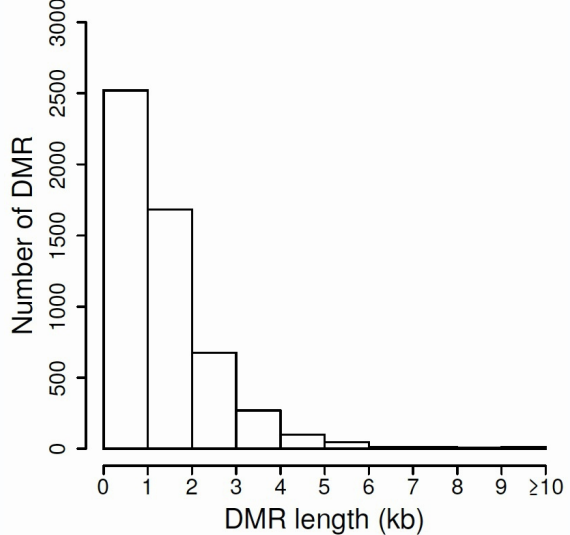
C P10 Spermatogonia vs Pachytene Spermatocytes



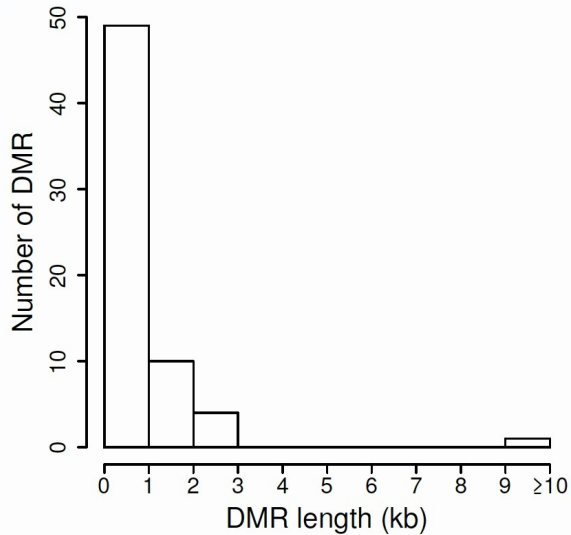
D Pachytene Spermatocytes vs Round Spermatids



E Round Spermatids vs Caput Epididymal Spermatozoa

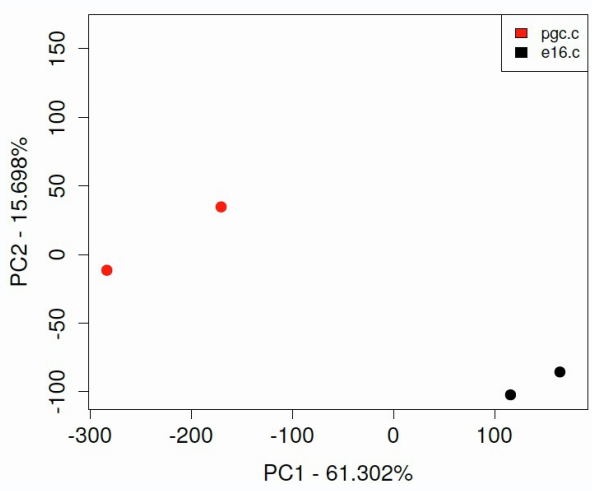


F Caput Epididymal Spermatozoa vs Cauda Sperm

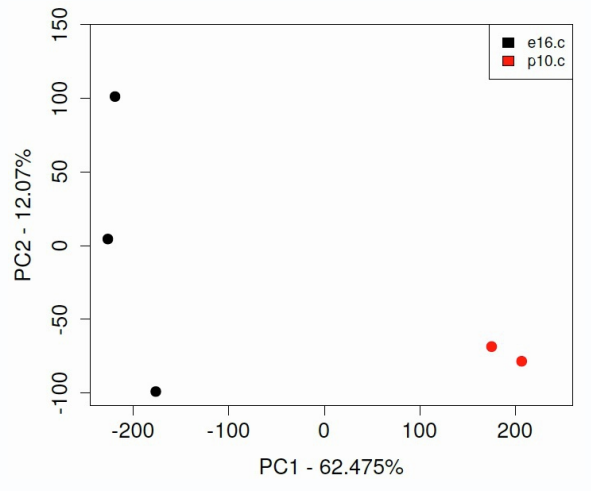


Supplemental Figure S5 (Color)

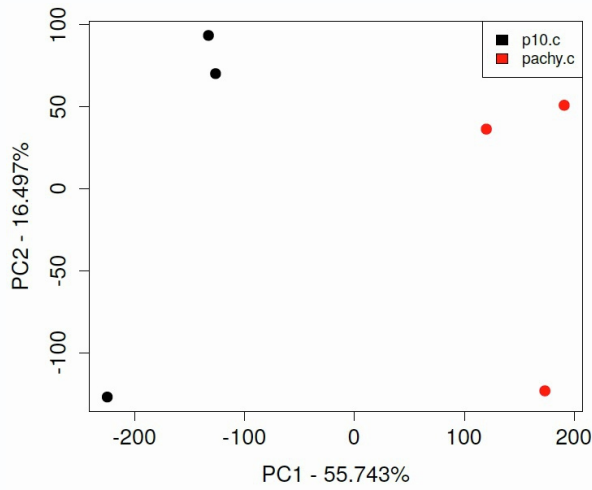
A E13 Primordial Germ Cells vs E16 Prospermatogonia



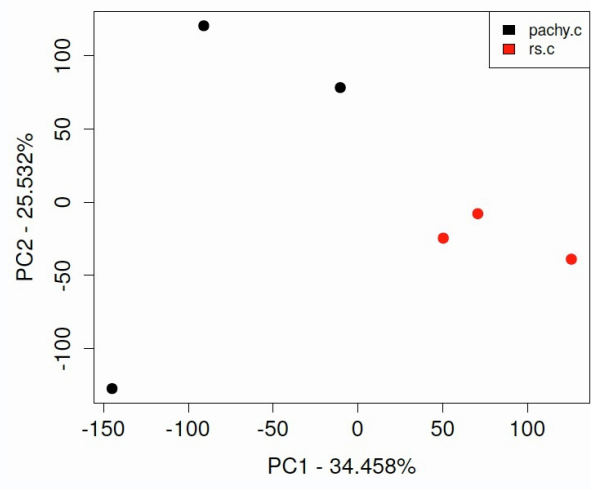
B E16 Prospermatogonia vs P10 Spermatogonia



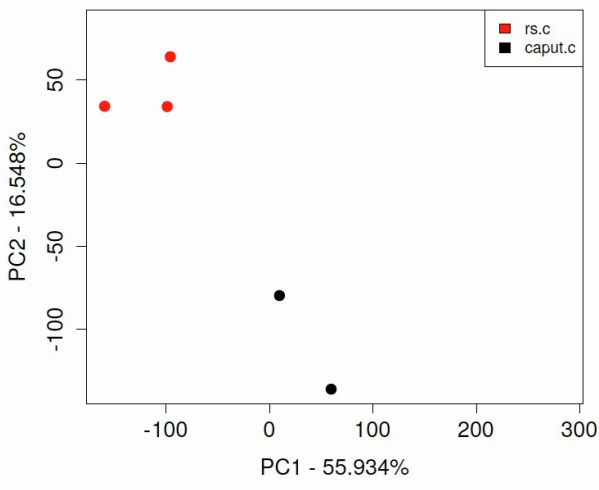
C P10 Spermatogonia vs Pachytene Spermatocytes



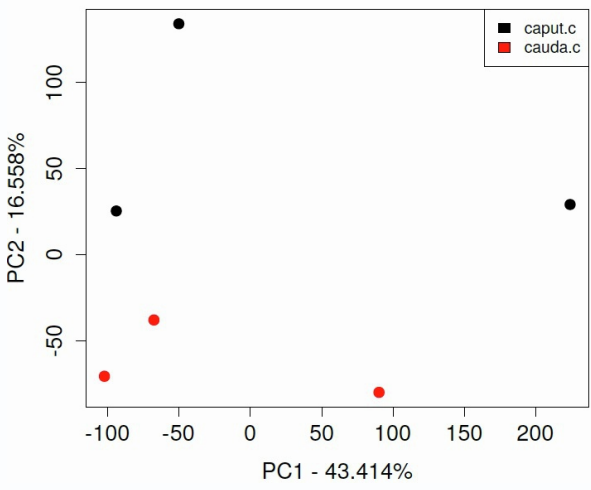
D Pachytene Spermatocytes vs Round Spermatids



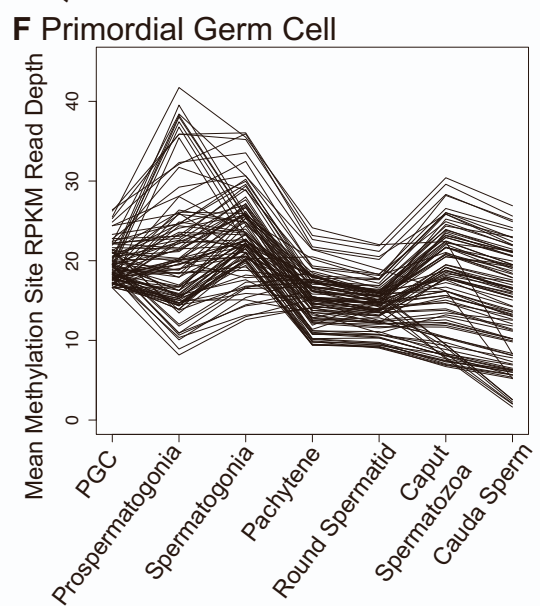
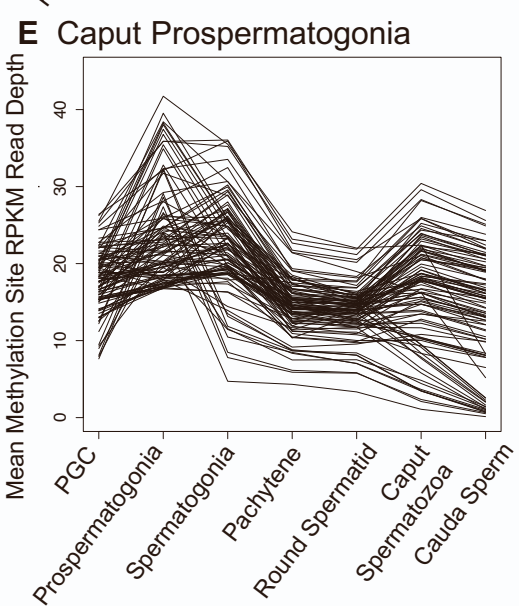
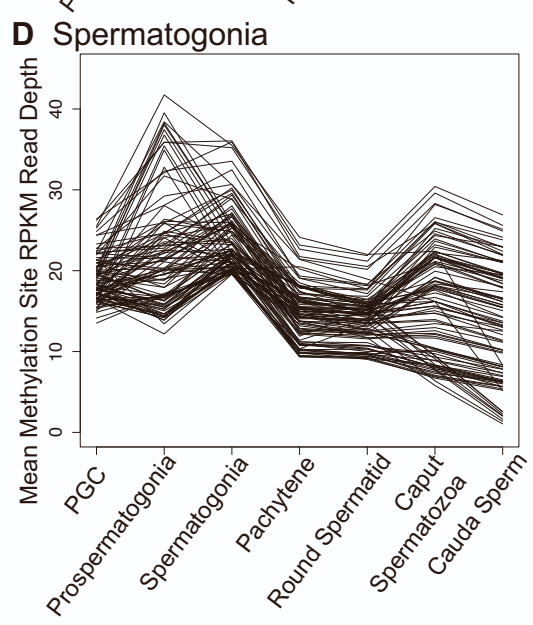
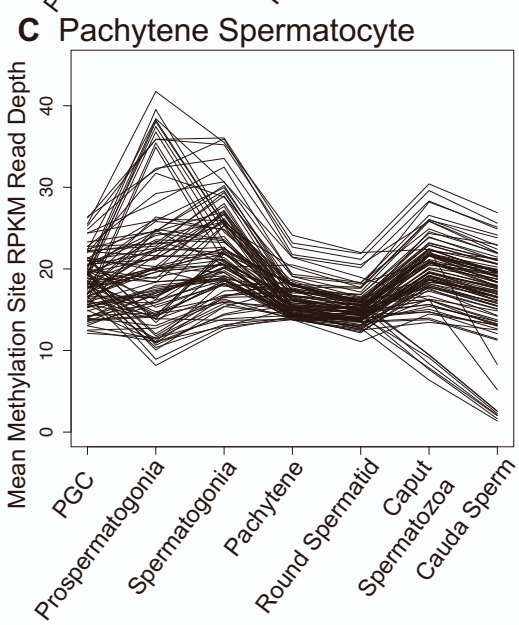
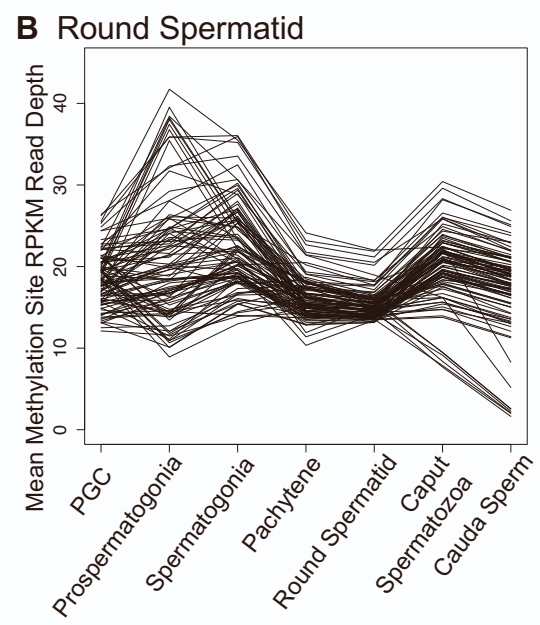
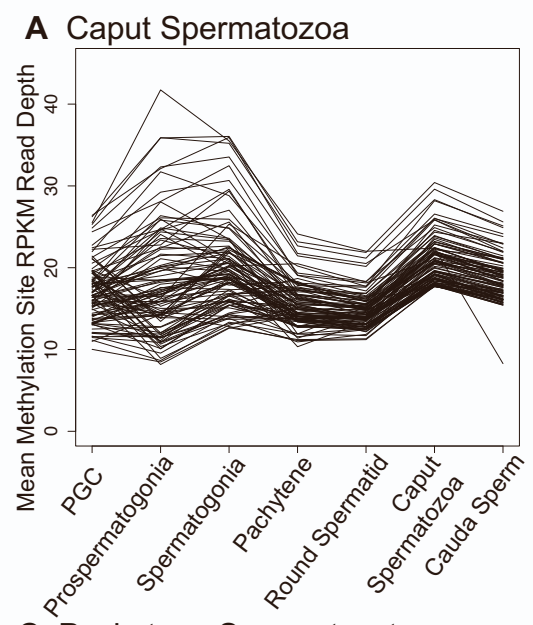
E Round Spermatids vs Caput Epididymal Spermatozoa



F Caput Epididymal Spermatozoa vs Cauda Spermatozoa



Top 100 Developmental Stage Methylation Sites Read Depth (RPKM) Comparisons



Supplemental Figure S7

DMR Associated Gene Pathways

KEGG Gene Pathways

PGC vs. Prospermatogonia

rno01100 Metabolic pathways (rat) (35)
rno04740 Olfactory transduction (rat) (20)
rno05200 Pathways in cancer (rat) (18)
rno05165 Human papillomavirus infection (rat) (14)
rno04934 Cushing syndrome (rat) (10)

Prospermatogonia vs. Spermatogonia

rno04740 Olfactory transduction (rat) (29)
rno01100 Metabolic pathways (rat) (20)
rno04080 Neuroactive ligand-receptor interaction (rat) (9)
rno05170 Human immunodeficiency virus 1 infection (rat) (9)
rno04010 MAPK signaling pathway (rat) (8)

Spermatogonia vs. Pachytene

rno01100 Metabolic pathways (rat) (23)
rno05200 Pathways in cancer (rat) (18)
rno05165 Human papillomavirus infection (rat) (15)
rno04151 PI3K-Akt signaling pathway (rat) (14)
rno04360 Axon guidance (rat) (13)

Pachytene vs. Round Spermatids

rno01100 Metabolic pathways (rat) (27)
rno05200 Pathways in cancer (rat) (17)
rno04151 PI3K-Akt signaling pathway (rat) (17)
rno04010 MAPK signaling pathway (rat) (14)
rno05165 Human papillomavirus infection (rat) (13)

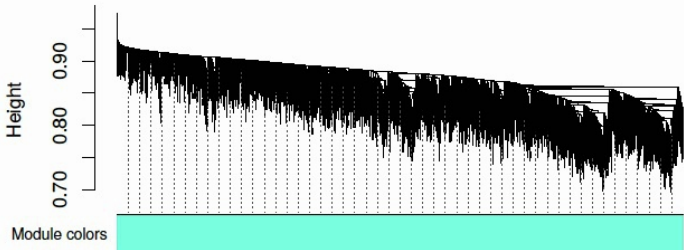
Round Spermatids vs. Caput

rno01100 Metabolic pathways (rat) (23)
rno05165 Human papillomavirus infection (rat) (19)
rno05163 Human cytomegalovirus infection (rat) (18)
rno04151 PI3K-Akt signaling pathway (rat) (18)
rno05200 Pathways in cancer (rat) (18)

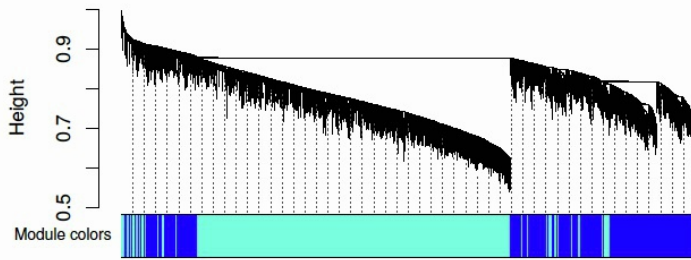
Caput vs. Cauda

rno01100 Metabolic pathways (rat) (42)
rno05205 Proteoglycans in cancer (rat) (14)
rno04360 Axon guidance (rat) (13)
rno05200 Pathways in cancer (rat) (12)
rno04740 Olfactory transduction (rat) (12)

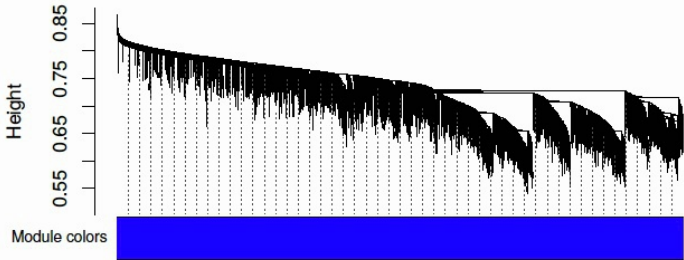
Cluster Dendrogram



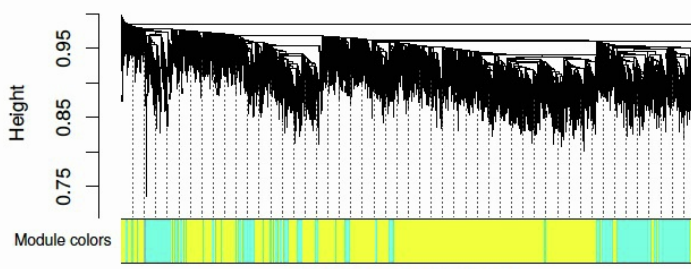
Cluster Dendrogram



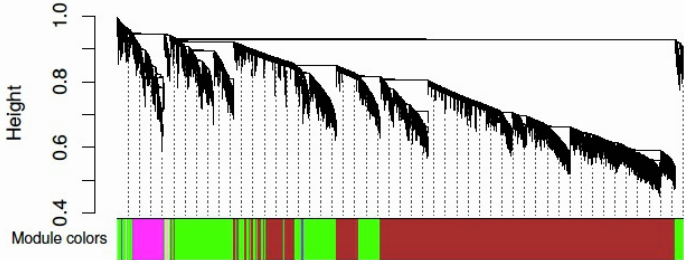
Cluster Dendrogram



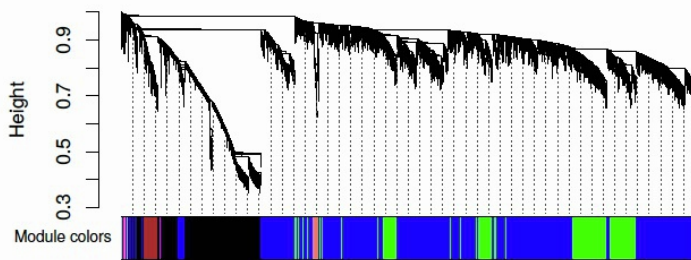
Cluster Dendrogram



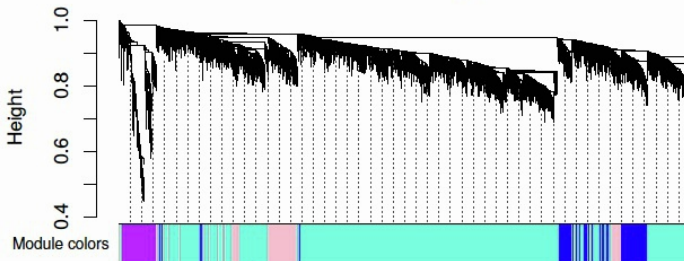
Cluster Dendrogram



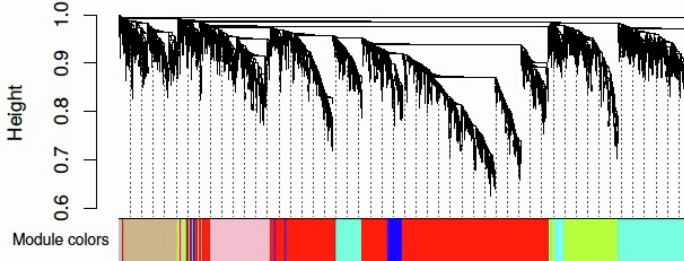
Cluster Dendrogram



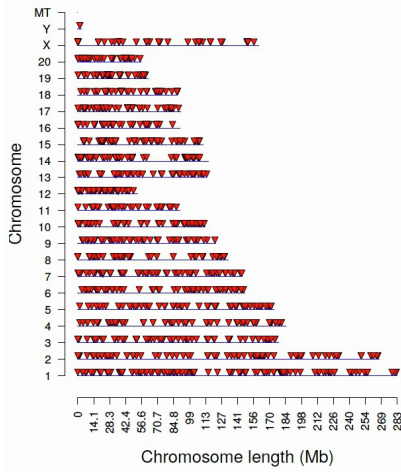
Cluster Dendrogram



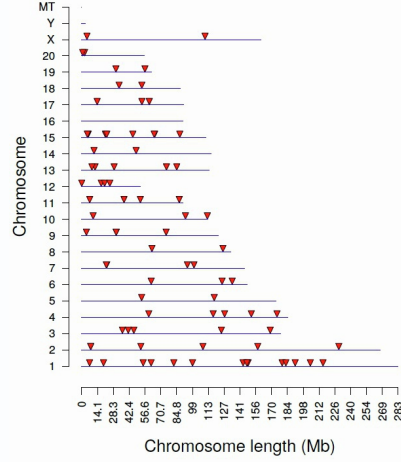
Cluster Dendrogram



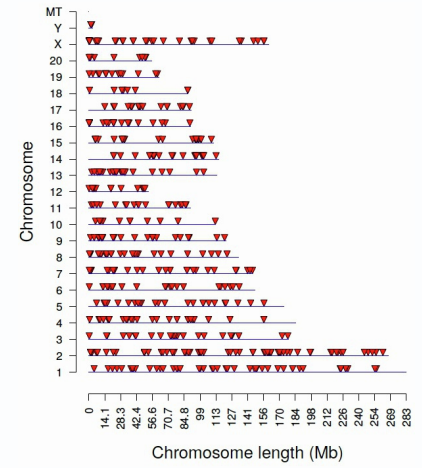
(A) Black



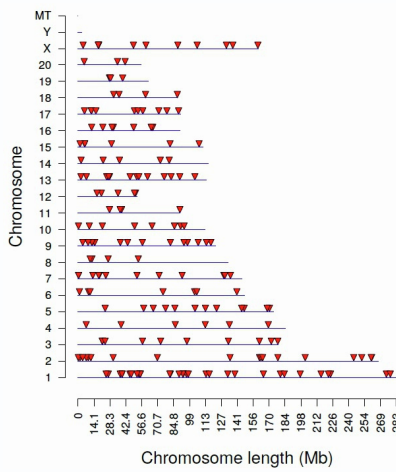
(B) Cyan



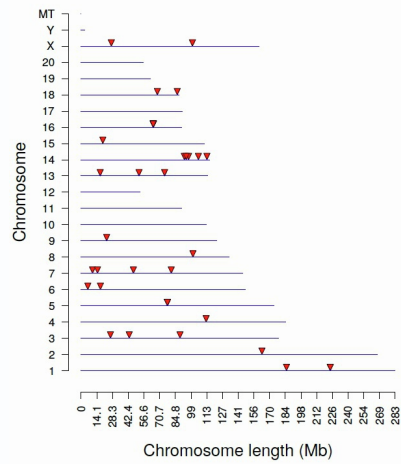
(C) Green Yellow



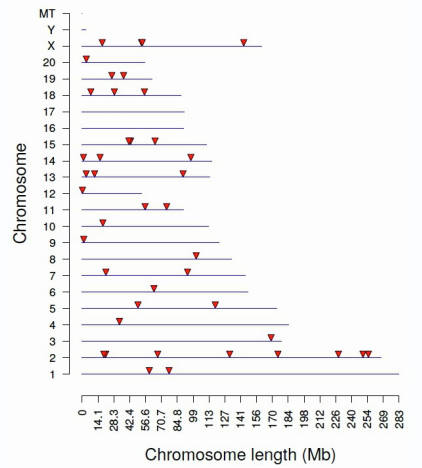
(D) Grey



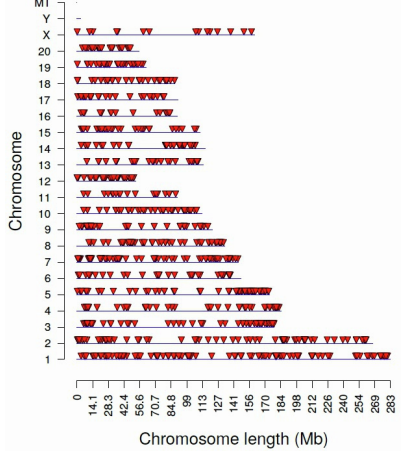
(E) Grey 60



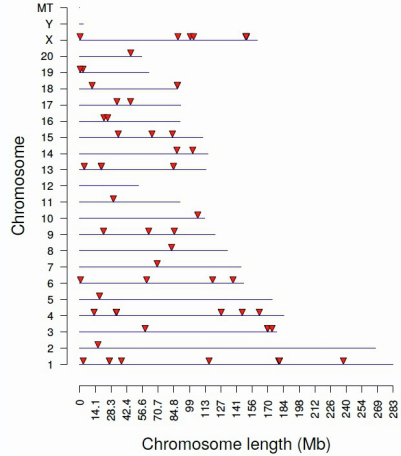
(F) Light Cyan



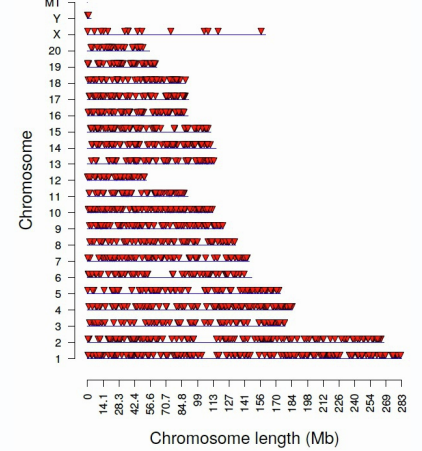
(G) Magenta



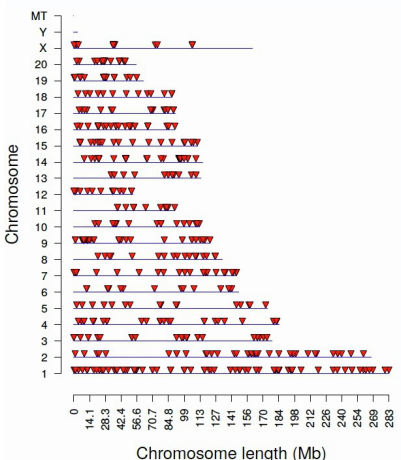
(H) Midnight Blue



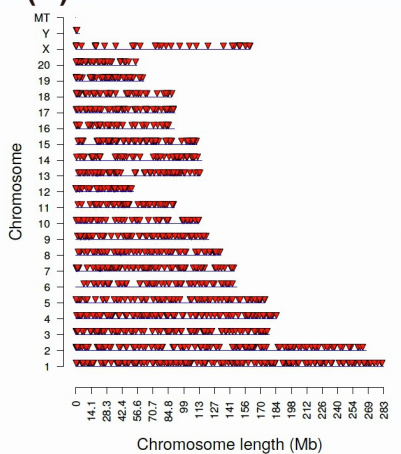
(I) Pink



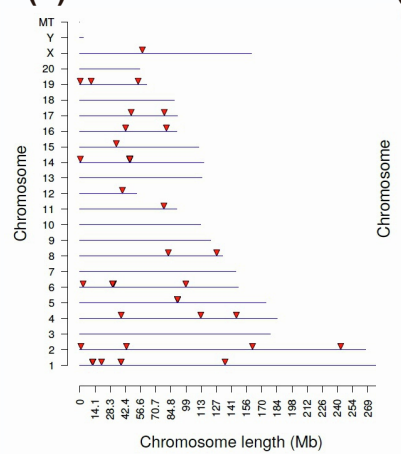
(J) Purple



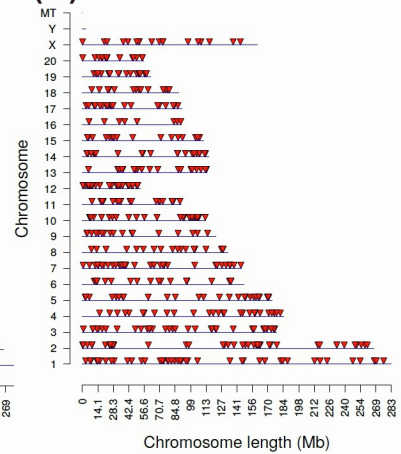
(K) Red



(L) Salmon



(M) Tan



WGCNA DNA Methylation Modules Sites Overlap with DMRs from Various Developmental Stage Comparisons

	PGC vs. E16	E16 vs. P10	P10 vs. Pachy	Pachy vs. RS	RS vs. Caput	Caput vs. Cauda
Midnight Blue	18	1	0	0	0	0
Salmon	31	0	0	0	17	0
Grey 60	2	0	0	0	0	0
Blue	668	6992	533	21	904	17
Turquoise	1833	11917	379	60	12	0
Magenta	138	6	5	0	13	0
Brown	435	2290	606	1	704	0
Green	26	31	141	0	315	0
Red	604	451	84	3	9	0
Pink	90	1	191	3	2	0
Purple	5	1	15	0	1	0
Light Cyan	0	0	7	0	0	0
Yellow	23	144	210	11	0	0
Black	239	505	1158	3	70	2
Tan	0	95	71	0	0	0
Cyan	0	24	1	0	0	0
Green Yellow	122	116	3	2	1	0
Grey	0	0	0	0	0	0