Supplementary Material

1 Supplementary scripts

The Reduced and Enlarged pools are processed in the scripts below, but the exact same scripts were used to compare the Control pools.

1.1 Bash - Quality control and trimming

```
### Quality control
git clone https://gitlab.bioinf.nioo.knaw.nl/tutorials/QC-NGS.git
source activate QC-NGS
fastqc -o ./reports/raw/ -t 12 ./data/raw/*.fastq.gz
cd ../../fastq_screen_v0.11.1/
./fastq_screen --bisulfite --outdir ../reports/raw-screen/ ../data/raw/*.fastq.gz
cd ..
multiqc -d -n report_all ./reports

### Trimming
source activate trim-galore
trim_galore -q 20 --length 36 --fastqc --keep --rrbs -o
/mnt/nfs/bioinfdata/home/NIOO/bernices/Projects/RRBS_2016_2018/RRBS_2016/QC-
NGS/data/clean_new/
/mnt/nfs/bioinfdata/home/NIOO/bernices/Projects/RRBS_2016_2018/RRBS_2016/QC-
NGS/data/raw/*.fastq.gz
```

1.2 Bash – Alignment and methylation calling

```
### Alignment
source activate bs-seeker2
python2.7 BSseeker2-master/bs_seeker2-build.py -f GCF_001522545.3_Parus_major1.1_genomic.fna --
aligner=bowtie2 -r
python2.7 ~/software//BSseeker2-master/bs_seeker2-align.py -i
~/Projects/RRBS_2016_2018/RRBS_2016/QC-
NGS/data/clean_new/rawPool1_GCCAATAT_L00M_R1_001_trimmed.fq --aligner=bowtie2 -o
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool1_bs2.bam -g
~/reference_genome/GCF_001522545.3_Parus_major1.1_genomic.fna --bt-p 12 --db ~/software/BSseeker2-
master/bs_utils/reference_genomes/Other/ -r --bt2--end-to-end
python2.7 ~/software//BSseeker2-master/bs_seeker2-align.py -i
~/Projects/RRBS_2016_2018/RRBS_2016/QC-
NGS/data/clean_new/rawPool2_CTTGTAAT_L00M_R1_001_trimmed.fq --aligner=bowtie2 -o
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool2_bs2.bam -g
~/reference_genome/GCF_001522545.3_Parus_major1.1_genomic.fna --bt-p 12 --db ~/software/BSseeker2-
master/bs_utils/reference_genomes/Other/ -r --bt2--end-to-end

### Methylation calling
python2.7 ~/software//BSseeker2-master/bs_seeker2-call_methylation.py -i
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool1_bs2.bam --CGmap
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool1_bs2_methcall.CGmap --
ATCGmap
```

```
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool1_bs2_methcall.ATCGmap
--db ~/software/BSseeker2-
master/bs_utils/reference_genomes/Other/GCF_001522545.3_Parus_major1.1_genomic.fna_rrbs_20_500_
bowtie2/
python2.7 ~/software//BSseeker2-master/bs_seeker2-call_methylation.py -i
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool2_bs2.bam --CGmap
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool2_bs2_methcall.CGmap --
ATCGmap
~/Projects/RRBS_2016_2018/RRBS_2016/bs2_data_2016/end_to_end/2016_Pool2_bs2_methcall.ATCGmap
--db ~/software/BSseeker2-
master/bs_utils/reference_genomes/Other/GCF_001522545.3_Parus_major1.1_genomic.fna_rrbs_20_500_
bowtie2/
```

1.3 Bash - Reformatting

```
grep "CG" 2016_Pool1_bs2_methcall.CGmap > 2016_Pool1_bs2_methcall_contextCG.CGmap
grep "CG" 2016_Pool2_bs2_methcall.CGmap > 2016_Pool2_bs2_methcall_contextCG.CGmap
cat 2016_Pool1_bs2_methcall_contextCG.CGmap | awk '{print $1,"\t",$3,"\t",$8,"\t",$2,"\t",$6}' >
2016_Pool1_bs2_methcall_contextCG_sorted.CGmap
cat 2016_Pool2_bs2_methcall_contextCG.CGmap | awk '{print $1,"\t",$3,"\t",$8,"\t",$2,"\t",$6}' >
2016_Pool2_bs2_methcall_contextCG_sorted.CGmap
cat 2016_Pool1_bs2_methcall_contextCG_sorted.CGmap | sed 's/G/-/' | sed 's/C/+/2' >
2016_Pool1_contextCG_methylkit
cat 2016_Pool2_bs2_methcall_contextCG_sorted.CGmap | sed 's/G/-/' | sed 's/C/+/2' >
2016_Pool2_contextCG_methylkit
grep "^NC_" 2016_Pool1_contextCG_methylkit > NC_2016_Pool1_contextCG_methylkit
grep "^NW_" 2016_Pool1_contextCG_methylkit > NW_2016_Pool1_contextCG_methylkit
grep "^NC_" 2016_Pool2_contextCG_methylkit > NC_2016_Pool2_contextCG_methylkit
grep "^NW_" 2016_Pool2_contextCG_methylkit > NW_2016_Pool2_contextCG_methylkit
cat NW_2016_Pool1_contextCG_methylkit | sed 's/C/+/' > NW_2016_Pool1_contextCG_methylkit_new
cat NW_2016_Pool2_contextCG_methylkit | sed 's/C/+/' > NW_2016_Pool2_contextCG_methylkit_new
cat NC_2016_Pool1_contextCG_methylkit NW_2016_Pool1_contextCG_methylkit_new >
2016_Pool1_contextCG_methylkit_correct
cat NC_2016_Pool2_contextCG_methylkit NW_2016_Pool2_contextCG_methylkit_new >
2016_Pool2_contextCG_methylkit_correct
```

1.4 R script – Filtering and statistical analysis

```
### methylKit: install, reading and uniting files
library(devtools)
install_github("al2na/methylKit", build_vignettes=FALSE,
        repos=BiocManager::repositories(),
        dependencies=TRUE)
library(methylKit)
file.list=list("2016_Pool1_contextCG_methylkit_correct", "2016_Pool2_contextCG_methylkit_correct")
myobj=methRead(file.list,
        sample.id=file.list,
        assembly="Pm",
        treatment=c(1, 0), context="CpG", header=FALSE, resolution="base",
        pipeline=list(fraction=TRUE, chr.col=1, start.col=2, end.col=2, coverage.col=3, strand.col=4,
                freqC.col=5))
```

```
meth.min1=unite(myobj)

### Calculate methylation %
meth.min1$methylation1<-meth.min1$numCs1/meth.min1$coverage1 #pool1
meth.min1$methylation2<-meth.min1$numCs2/meth.min1$coverage2 #pool2

### Removing sites that are fully methylated or unmethylated in both pools
meth.min1_sub1<-meth.min1[!(meth.min1$methylation1==meth.min1$methylation2 &
meth.min1$methylation2==1),]
meth.min1_sub2<-meth.min1_sub1[!(meth.min1_sub1$methylation1==meth.min1_sub1$methylation2 &
meth.min1_sub1$methylation2==0),]

### unmerge pools
colnames(meth.min1_sub2)
myobj.sub1<-getData(meth.min1_sub2)[,c(1:2,5,4,11)]
myobj.sub2<-getData(meth.min1_sub2)[,c(1:2,8,4,12)]
library("readr")
write_tsv(myobj.sub1, path = "2016_Pool1_contextCG_methylkit_correct_nodouble01.txt")
write_tsv(myobj.sub2, path = "2016_Pool2_contextCG_methylkit_correct_nodouble01.txt")

### Filtering (10X coverage and percentile) and uniting pools
library(devtools)
library(methylKit)
file.list2=list("2016_Pool1_contextCG_methylkit_correct_nodouble01.txt",
"2016_Pool2_contextCG_methylkit_correct_nodouble01.txt")
myobj2=methRead(file.list2,
        sample.id=file.list2,
        assembly="Pm",
        treatment=c(1, 0), context="CpG", header=TRUE, resolution="base",
        pipeline=list(fraction=TRUE, chr.col=1, start.col=2, end.col=2, coverage.col=3, strand.col=4,
                freqC.col=5))
filtered.myobj=filterByCoverage(myobj2,lo.count=10,lo.perc=NULL,
                  hi.count=NULL,hi.perc=99.9)
filtered_normalized_myobj=normalizeCoverage(filtered.myobj)
meth.min=unite(filtered_normalized_myobj)

### Get significant sites
tiles=tileMethylCounts(filtered_normalized_myobj, win.size=500,step.size=500, cov.bases=3)
tiles=unite(tiles)
myDiff_min=calculateDiffMeth(meth.min, overdispersion=c("none"), adjust=c("bonferroni"), mc.cores=2)
write.table(x=myDiff_min, file=sprintf('myDiff_min_pool1_vs_pool2_2016'), quote=FALSE)
myDiff_tiles=calculateDiffMeth(tiles, overdispersion=c("none"), adjust=c("bonferroni"), mc.cores=2)
write.table(x=myDiff_tiles, file=sprintf('myDiff_tiles_pool1_vs_pool2_2016'), quote=FALSE)
myDiffp_min=getMethylDiff(myDiff_min,difference=25,qvalue=0.05)
write.table(x=myDiffp_min, file=sprintf('myDiff25_min_pool1_vs_pool2_2016'),
       quote=FALSE)
myDiffp_tiles=getMethylDiff(myDiff_tiles,difference=25,qvalue=0.05)
write.table(x=myDiffp_tiles, file=sprintf('myDiff25_tiles_pool1_vs_pool2_2016'),
       quote=FALSE)
```

1.5 R script – Plots

```
### Volcano plot, -log10(0.05/235618)=6.673238 (for the Control pools: -log10(0.05/213764)=6.630965)
install.packages("gridExtra")
library(gridExtra)
library(ggplot2)
fc=myDiff_min$meth.diff
sig=-log10(myDiff_min$pvalue)
df<-data.frame(fc,sig)
df$thre<-as.factor(abs(fc) >= 25 & sig > 6.673238)
ggplot(data=df, aes(x=fc, y=sig, color=thre)) +
  geom_point(alpha=1, size=0.75) +
  theme(legend.position = "none") +
  labs(title="B", y=expression("-log"^"10"*"(p-value)"), x = expression(Delta*"(% methylation level)")) +
scale_x_continuous(breaks=c(-75,-50,-25,0,25,50,75)) +
  theme(panel.background = element_rect(fill="white", colour="white", size=0.5, linetype="solid",
color="grey70")) +
  theme(panel.grid.major = element_line(colour="grey90", size=0.1,
linetype="blank",lineend="butt",color="grey90"),
        panel.grid.minor = element_line(colour="grey90", size=0.1,
linetype="blank",lineend="butt",color="grey90"),
        plot.title = element_text(face="bold", color="black", size=25, hjust = -0.1),
        axis.title.x = element_text(size=14),
        axis.title.y = element_text(size=14))+
  scale_color_manual(values = c("grey60","dodgerblue4")) +
  geom_hline(yintercept = 6.673238,color="black",linetype="dashed",alpha=0.7) +
  geom_vline(xintercept = -25,color="black",linetype="dashed",alpha=0.7) +
  geom_vline(xintercept = 25,color="black",linetype="dashed",alpha=0.7)

### Manhattan plot
library(devtools)
install_github("drveera/ggman")
library(ggman)
library("RColorBrewer")
devtools::install_url("https://github.com/wilkelab/cowplot/archive/0.6.3.zip")
library(cowplot)
chr_names<-
read.table(file="/mnt/nfs/bioinfdata/home/NIOO/bernices/reference_genome/GCF_001522545.3_Parus_m
ajor1.1_assembly_report_chr_names.txt",sep=" ",na.strings = "na")
colnames(chr_names)<-c("Name","Code")
library(plyr)
myDiff_min_ChrNamesShort<-myDiff_min
myDiff_min_ChrNamesShort$chrName<-mapvalues(x=myDiff_min_ChrNamesShort$chr,
                                            from=levels(chr_names$Code),
                                            to  =levels(chr_names$Name))
```

```
myDiff_min_ChrNamesShort$chrName_scaffolds<-as.character(myDiff_min_ChrNamesShort$chrName)
Scaffolds<-grep(pattern = "Scaffold",x = myDiff_min_ChrNamesShort$chrName)
myDiff_min_ChrNamesShort$chrName_scaffolds[Scaffolds]<-"scaffolds"
myDiff_min_ChrNamesShort$chrName_scaffolds<-
as.factor(myDiff_min_ChrNamesShort$chrName_scaffolds)
significant <-
myDiff_min_ChrNamesShort$start[myDiff_min_ChrNamesShort$pvalue<0.05/nrow(myDiff_min_ChrNamesS
hort) & abs(myDiff_min_ChrNamesShort$meth.diff)>=25]
p<-ggman(getData(myDiff_min_ChrNamesShort),
      chrom="chrName_scaffolds",
      snp = "start",
      pvalue = "pvalue",
      title = " ",
      sigLine = -log10(0.05/nrow(myDiff_min_ChrNamesShort)),
      pointsize = 1)
p<-p + theme(axis.text.x = element_text(angle = 90)) +
   theme(panel.background = element_rect(fill="white", colour="white", size=0.5, linetype="solid",
color="grey70")) +
   theme(panel.grid.major = element_line(colour="grey90", size=0.1,
linetype="solid",lineend="butt",color="grey90"),
       panel.grid.minor = element_line(colour="grey90", size=0.1,
linetype="blank",lineend="butt",color="grey90"))
p<-p + coord_cartesian(ylim = c(0, 20))
ggmanHighlight(ggmanPlot = p,highlight = significant, colour = "dodgerblue4",size=1) +
  labs(title="B", y = expression("-log"^"10"*"(p-value)"), x="Chromosome") +
  theme(axis.text.x = element_text(angle = 90), plot.title = element_text(face="bold", color="black", size=25,
hjust = -0.065))

### PCA all pools
library(devtools)
library(methylKit)

file.list=list("2016_Pool1_contextCG_methylkit_correct",
"2016_Pool2_contextCG_methylkit_correct","2018_Pool1_contextCG_methylkit_correct",
"2018_Pool2_contextCG_methylkit_correct")
myobj=methRead(file.list,
          sample.id=list("enlarged","reduced","ctrl1","ctrl2"),
          assembly="Pm",
          treatment=c(1,2,0,0), context="CpG", header=FALSE, resolution="base",
          pipeline=list(fraction=TRUE, chr.col=1, start.col=2, end.col=2, coverage.col=3, strand.col=4,
                    freqC.col=5))
meth.min1=unite(myobj)

PCASamples(meth.min1,screeplot=T,transpose=T,sd.filter=T)
PCA<-PCASamples(meth.min1,obj.return=TRUE,transpose=T,sd.filter=T,scale=T)

PCA$sdev
eigen<-(PCA$sdev)^2
Explained<-100*eigen/sum(eigen)
```

1.6 R script - Gene annotation

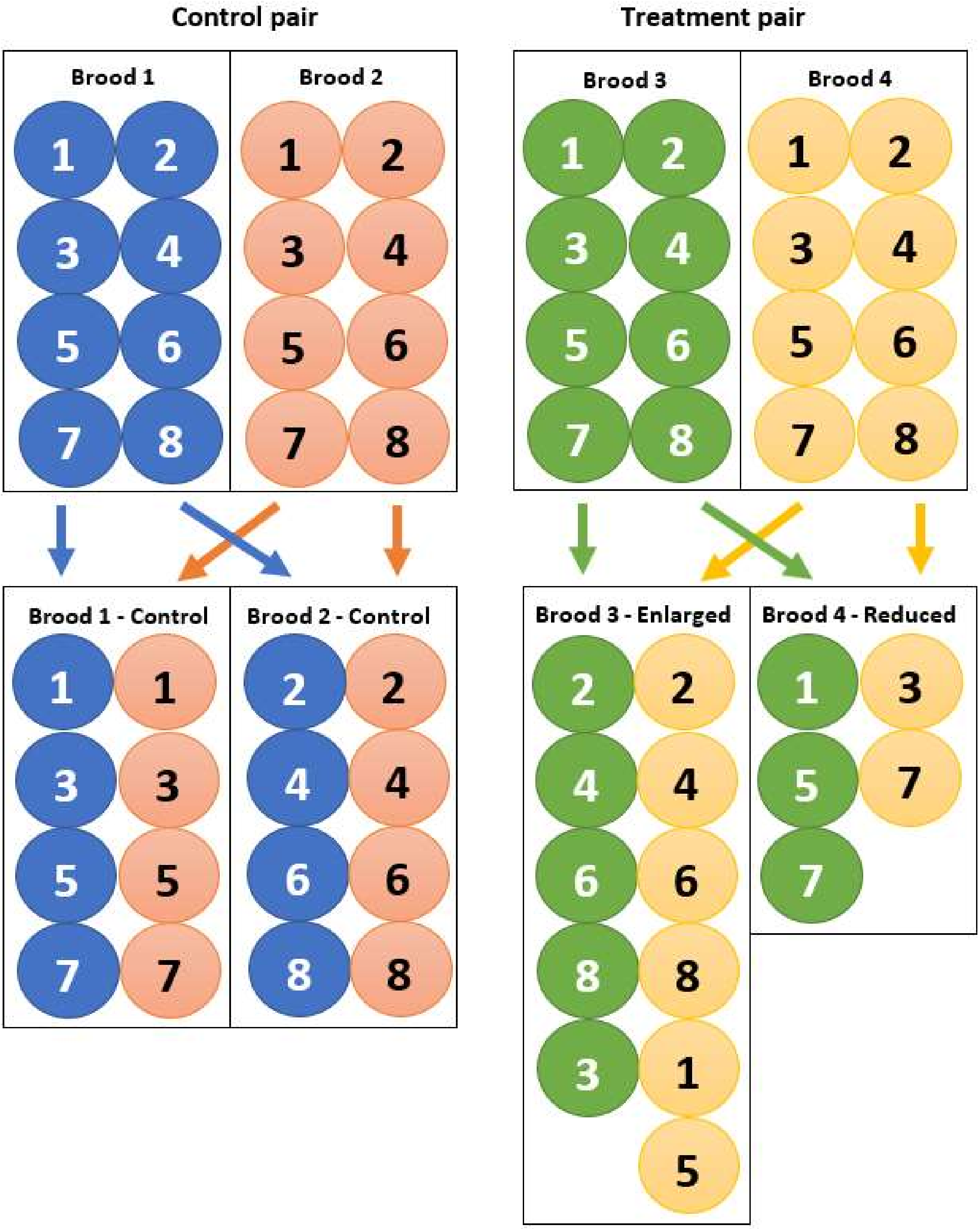
```
install.packages("remotes")
remotes::install_github("jasongraf1/JGmisc")
library(JGmisc)
detachAllPackages()
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install()
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install(c("GenomicFeatures", "rtracklayer"))
library(GenomicFeatures)
library(rtracklayer)
BiocManager::install(c("BSgenome", "BiocGenerics"))
library(BiocGenerics)
library(BSgenome)

## Genome info
ParusM_1.1_genome <- read.table("chr.length.txt", header=FALSE, sep="\t")
colnames(ParusM_1.1_genome)<-c("chrom","length")
gff <- makeTxDbFromGFF("GCF_001522545.3_Parus_major1.1_genomic.gff", format="gff3",
organism="Parus major", chrominfo=ParusM_1.1_genome)

## Get annotations
PROmoters_2k_new <- promoters(gff, upstream=2000, downstream=200, columns=c("tx_name", "gene_id"))

# promoter length
PROmoters_2k.t <- trim(PROmoters_2k_new, use.names=TRUE)
TSS.laine <- promoters(gff, upstream=300, downstream=50, columns=c("tx_name", "gene_id")) # TSS as in
Laine et al., 2016. Nature Communications
TSS.laine.t <- trim(TSS.laine, use.names=TRUE)
downstream.laine <- flank(genes(gff), 10000, start=FALSE, both=FALSE, use.names=TRUE)
downstream.laine.t <- trim(downstream.laine, use.names = TRUE)
upstream.laine <- promoters(genes(gff), upstream=10000, downstream=0)
upstream.laine.t <- trim(upstream.laine, use.names = TRUE)

## Exons group by gene
exons.transcript <- exonsBy(gff, "tx", use.names=TRUE) # all exons for each transcript
exons.gene <- exonsBy(gff, "gene") # group exons by genes
exons.transcript.unlist <- unlist(exons.transcript)
exons.gene.unlist <- unlist(exons.gene)
## Introns grouped by transcript
introns.transcripts <- intronsByTranscript(gff, use.names=TRUE)
introns.transcripts.unlist <- unlist(introns.transcripts)
```

```
## export files
export(genes(gff), "genes.gff3")
export(PROmoters_2k.t, "promoters_2k.t.gff3")
export(TSS.laine.t, "TSS.laine.t.gff3")
export(downstream.laine.t, "downstream.laine.t.gff3")
export(upstream.laine.t, "upstream.laine.t.gff3")
export(exons.transcript.unlist, "exons.transcript.gff3")
export(exons.gene.unlist, "exons.gene.gff3")
export(introns.transcripts.unlist, "introns.transcripts.gff3")
```

1.7 Bash – Bisulfite conversion

```
source activate samtools_1.9
samtools view 2016_Pool1_bs2.bam | grep "XS:i:0" | wc -l
samtools view 2016_Pool1_bs2.bam | grep "XS:i:1" | wc -l
samtools view 2016_Pool2_bs2.bam | grep "XS:i:0" | wc -l
samtools view 2016_Pool2_bs2.bam | grep "XS:i:1" | wc -l
```

2 Supplementary Figures and Tables



Supplementary Figure 1 | Schematic overview of the cross-fostering procedure and the brood size manipulation for both a control pair and a treatment pair. Each circle represents a nestling. Each color represents the brood of origin. Each number represents the weight rank of a nestling within the brood of origin. On the top four unmanipulated broods are shown, two are part of a control pair and two are part of a treatment pair. The part below the arrows shows the final distribution of the nestlings over the broods (after cross-fostering and brood size manipulation).

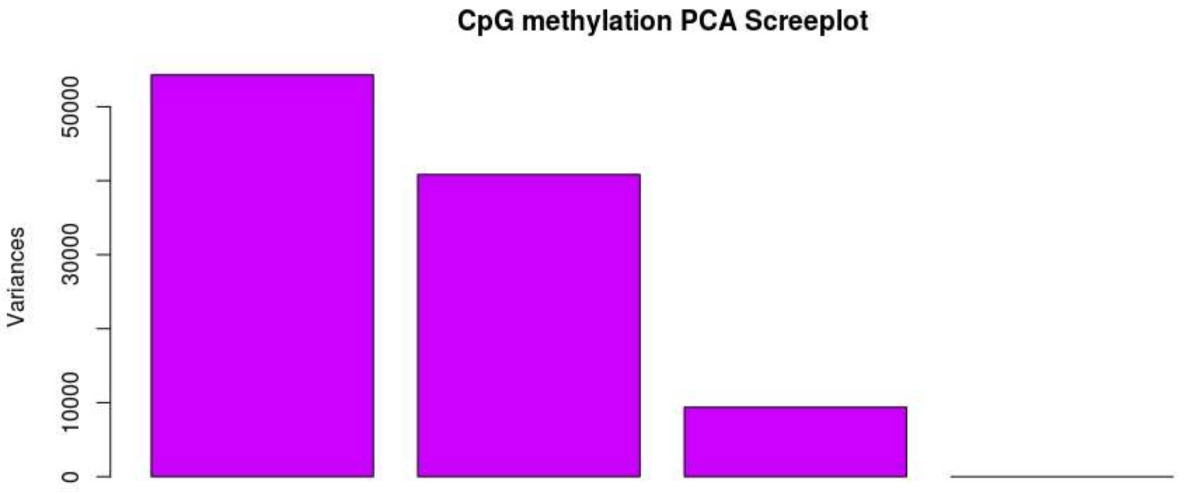
Supplementary Table 1 | Overview of brood of rearing, brood of rearing, treatment and pool for every individual nestling in this study.

Individual	Brood of origin	Brood of rearing	Treatment	Pool
S1-256	256	237	Enlarged	Enlarged
S2-256	256	256	Reduced	Reduced
S1-251	251	251	Enlarged	Enlarged
S2-251	251	285	Reduced	Reduced
S1-257	257	257	Enlarged	Enlarged
S2-257	257	246	Reduced	Reduced
S1-239	239	307	Enlarged	Enlarged
S2-239	239	239	Reduced	Reduced
S1-355	355	355	Enlarged	Enlarged
S2-355	355	212	Reduced	Reduced
S1-280	280	360	Enlarged	Enlarged
S2-280	280	280	Reduced	Reduced
S1-375	375	362	Enlarged	Enlarged
S2-375	375	375	Reduced	Reduced
S1-278	278	278	Control	Control2
S2-278	278	369	Control	Control1
S1-297	297	297	Control	Control2
S2-297	297	306	Control	Control1
S1-300	300	300	Control	Control1
S2-300	300	350	Control	Control2
S1-306	306	306	Control	Control1
S2-306	306	297	Control	Control2
S1-350	350	350	Control	Control2
S2-350	350	300	Control	Control1
S1-369	369	369	Control	Control1
S2-369	369	278	Control	Control2

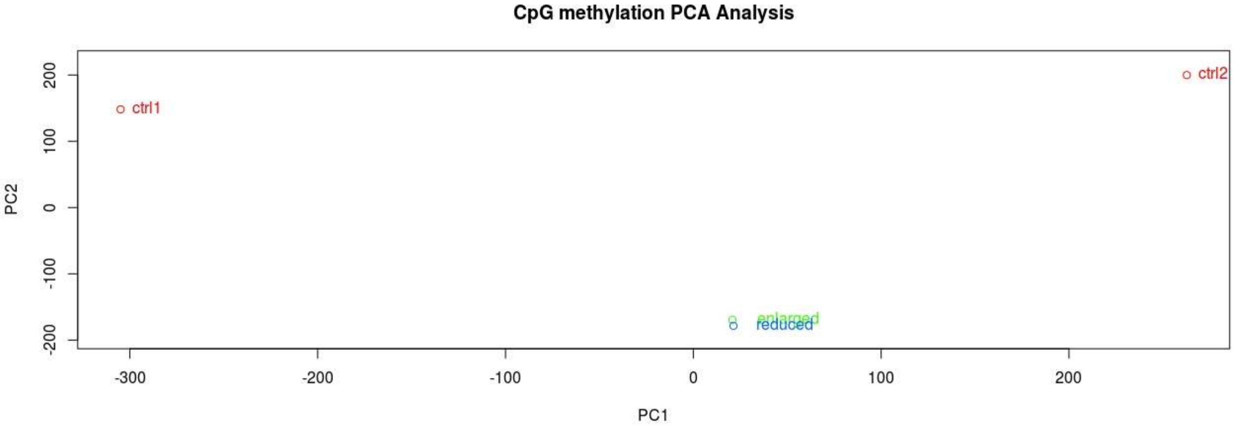
Supplementary Table 2 | Number and percentage of fully bisulfite converted reads, number and percentage of non-fully bisulfite converted reads and total number of reads after read trimming and mapping.

Pool	# of converted reads	percentage of converted reads*	# of non-fully converted reads	percentage of non-fully converted reads	# total reads aligned with unique best hit
Control1	15,590,746	99.997%	504	0.003%	15,591,250
Control2	19,932,757	99.998%	482	0.002%	19,933,239
Enlarged	134,095,521	99.991%	12.624	0.009%	134,108,145
Reduced	117,038,927	99.993%	8.317	0.007%	117,047,244

*The % of converted against unconverted cytosines outside of a CpG (hence CH) context was used. BS-Seeker2 provides all reads with an XS tag. If the number of methylated CH sites divided by all CH sites exceeds 0.5 and the number of methylated CH sites in a read exceeds 5, then an XS:i:1 tag is given. This indicates a higher-than-threshold number of CH sites in a read are not converted and hence that the read is recognized as "not fully converted". Reads below the thresholds are given the tag XS:i:0 and are recognized as fully converted. The percentage of fully converted reads was calculated as the number of fully converted reads divided by the total number of reads.



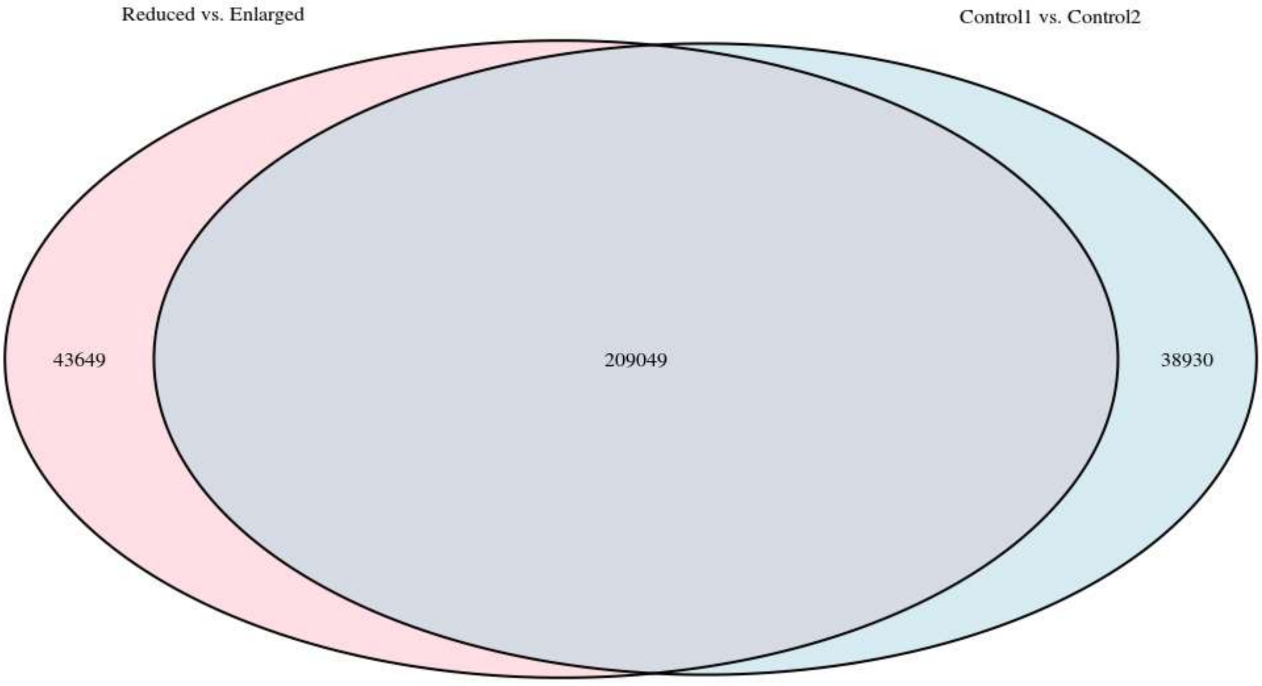
Supplementary Figure 2 | PCA scree plot showing the importance of components. PCA included DNA methylation levels of CpG sites present in all pools (Enlarged, Reduced, Control1 and Control2) with minimally 10x coverage. Of the total variance, 51.95% is explained by PC1, 39.07% by PC2, 8.98% by PC3 and 2.20 × 10-29 % by PC4.



Supplementary Figure 3 | PCA plot showing clustering of the four pools (Enlarged, Reduced, Control1 and Control2) based on DNA methylation levels of CpG sites present in all pools with minimally 10x coverage. Clustering along PC1 (eigenvalue 54303.54) and PC2 (eigenvalue 40836.68) is shown.

Supplementary Table 3 | Number of reads before and after read trimming with mapping and methylation calling success of BS-Seeker2. CpG site filtering was done with methylKit.

Pool	**# of reads**	**# of reads after filtering**	**GC**	**Mapping efficiency**	**# of reads aligned with unique best hit**	**CpG's before filtering**	**CpG's after 10x filtering**	**CpG's after 0% or 100% methylation filtering and uniting**	**Shared CpG's after percentile filtering and uniting**
Control1	27,252,618	27,249,741	37%	57.22%	15,591,250	360,116	257,527	213,997	213,764
Control2	35,267,028	35,264,858	37%	56.52%	19,933,239	363,942	264,387		
Enlarged	311,518,306	240,326,160	37%	55.80%	134,108,145	464,306	262,425	235,868	235,618
Reduced	264,636,306	208,157,145	36%	56.23%	117,047,244	476,669	256,303		



Supplementary Figure 4 | Venn diagram showing an overlap of 209,049 CpG sites between all four pools (Reduced, Enlarged, Control1 and Control2).

Supplementary Table 4 | Number of DMS between pool Control1 and pool Control2 and between the Reduced pool and the Enlarged pool.

	Control1 vs. Control2	**Reduced vs. Enlarged**
Total	17	32
No annotation	3	9
Annotated	14	23
Promoter	4	9
of which TSS	2	3
Exon	1	3
Intron	5	8
Downstream	2	2
Upstream	1	1
Downstream and upstream	1	0

Supplementary Table 5 | Location, chromosome number or unplaced scaffold, p-value, Δ of % methylation level, region and gene name associated with DMS between Control1 pool and Control2 pool with minimally 25% differential methylation.

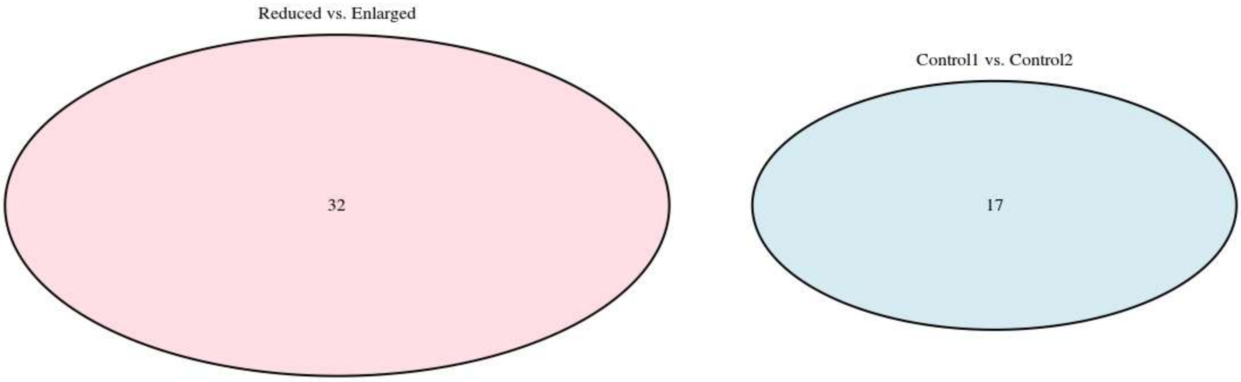
Location DMS	**Chrom./scaf.**	**P-value**	**Δ Meth.**	**Region**	**Gene**
113634610	chr1	4.24×10^{-08}	71%	-	-
9668340	chr4A	1.61×10^{-10}	47%	-	-
54944183	chr5	1.68×10^{-08}	26%	promoter	HSPA2
60057909	chr5	1.88×10^{-07}	77%	downstream	ARF6
17727814	chr7	2.12×10^{-07}	28%	promoter	METTL8
37135105	chr7	1.87×10^{-09}	62%	downstream	CYTIP
4427330	chr10	2.30×10^{-08}	32%	intron	RORA
14791064	chr14	1.46×10^{-07}	71%	exon	LOC107211364
2212920	chr17	5.56×10^{-08}	80%	intron	KCNT1
919719	chr18	2.57×10^{-08}	85%	downstream	CHMP6
				upstream	BAIAP2
7556239	chr19	5.88×10^{-08}	71%	upstream	TBX2
1171186	chr20	1.57×10^{-07}	-75%	-	-
6759952	chr20	8.15×10^{-08}	28%	TSS	LOC107213098
200856	chr22	9.46×10^{-20}	34%	intron	TGOLN2
11977179	chrZ	5.05×10^{-11}	25%	TSS	LOC107198170
68877019	chrZ	2.42×10^{-10}	37%	intron	APC
175111	scaffold	7.77×10^{-08}	33%	intron	ATF7

- *Not annotated*

Supplementary Table 6 | Location, chromosome number or unplaced scaffold, p-value, Δ of % methylation level, region and gene name associated with DMS between the Enlarged pool and the Reduced pool with minimally 25% differential methylation.

Location DMS	**Chrom./scaf.**	**P-value**	**Δ Meth.**	**Region**	**Gene**
75729799	chr2	5.62×10^{-10}	-27%	intron	CDH18
149822731	chr2	4.28×10^{-9}	34%	TSS	TSTA3
13870784	chr4	1.04×10^{-13}	72%	intron	PRKG2
564313	chr4A	4.99×10^{-17}	-26%	-	-
32813644	chr6	7.27×10^{-9}	27%	-	-
1441126	chr14	6.32×10^{-8}	-31%	upstream	PPL
15624191	chr14	1.86×10^{-9}	-25%	exon	ERN2
13816573	chr15	1.10×10^{-9}	-30%	exon	POMC
4729995	chr17	1.42×10^{-14}	-26%	intron	LAMC3
4730000	chr17	7.06×10^{-11}	-26%	intron	LAMC3
4730148	chr17	1.42×10^{-17}	-26%	exon	LAMC3
2363952	chr18	1.66×10^{-8}	29%	downstream	LOC107212597
1797839	chr21	1.17×10^{-12}	-45%	promoter	LOC107213704
730012	chr22	1.36×10^{-13}	70%	promoter	PROM2
5854480	chr23	6.54×10^{-10}	-27%	-	-
6296431	chr23	5.72×10^{-8}	-29%	-	-
6427084	chr23	3.46×10^{-12}	37%	downstream	LOC107214194
597551	chr25LG2	1.89×10^{-8}	-25%	intron	TARS2
5506639	chr26	2.27×10^{-8}	26%	intron	PLXNA2
70917608	chrZ	4.27×10^{-8}	26%	TSS	LOC107198385
506	scaffold	4.22×10^{-8}	-26%	-	-
913	scaffold	4.13×10^{-9}	41%	-	-
1719	scaffold	1.70×10^{-8}	-31%	-	-
1772	scaffold	6.52×10^{-8}	28%	promoter	LOC107199333
4319	scaffold	1.70×10^{-8}	58%	-	-
11100	scaffold	4.62×10^{-8}	41%	-	-
11884	scaffold	1.49×10^{-10}	-53%	promoter	WDR83OS
19200	scaffold	4.18×10^{-8}	29%	TSS	LOC107199222
50860	scaffold	1.82×10^{-14}	-34%	promoter	PREB
66988	scaffold	6.78×10^{-10}	-26%	intron	SCRN2
73459	scaffold	6.10×10^{-10}	25%	intron	LOC107198895
246819	scaffold	1.77×10^{-7}	-29%	promoter	ATF6

- *Not annotated*



Supplementary Figure 5 | Venn diagram showing 32 DMS between the Enlarged pool and the Reduced pool, 17 DMS between Control1 pool and Control2 pool and an overlap of zero DMS between the two comparisons.

Supplementary Table 7 | Number of DMS between Control1 pool and Control2 pool and between the Enlarged pool and the Reduced pool (minimally 25% differential methylation), number of DMS found in one comparison that is also found in het other comparison and number and percentage of DMS found in one comparison that is covered in the other comparison.

Comparison	**# DMS**	**# overlapping DMS in other comparison**	**# covered in other comparison**	**percentage covered in other comparison**
Control1 vs. Control2	17	0	11	65%
Reduced vs. Enlarged	32	0	26	81%

Supplementary Table 8 | Gene name, full name and biological functions associated with DMS between Control1 pool and Control2 pool with minimally 25% differential methylation.

Gene name	**Full name**	**Summary of GO terms (mainly biological process)**
HSPA2	Heat shock protein family A (Hsp70) member 2	Protein refolding, spermatogenesis
METTL8	Methyltransferase like 8	Skeletal muscle tissue development, fat cell differentiation, histone acetylation, mRNA methylation
LOC107198170	Oncostatin-M-specific receptor subunit beta-like	Cell population proliferation, inflammatory response
LOC107213098	Zinc finger SWIM domain-containing protein 1-like	Zinc and metal ion binding
LOC107211364	Testis-expressed sequence 2 protein-like	-
ATF7	Activating transcription factor 7	Regulation transcription
APC	APC regulator of WNT signaling pathway	Cell proliferation (negative), tissue formation, cell migration
TGOLN2	Trans-golgi network protein 2	Post-translational protein modification, cellular protein metabolic process, membrane organization (exocytic vesicle formation)
KCNT1	Potassium sodium-activated channel subfamily T member 1	Potassium ion transport (neuronal excitability)
RORA	RAR related orphan receptor A	Transcriptional regulation, maintenance circadian rhythm, development of cerebellum, fat cell differentiation, muscle cell development, immune response

- *Function could not be verified*

Supplementary Table 9 | Gene name, full name and biological functions associated with DMS between the Enlarged pool and the Reduced pool with minimally 25% differential methylation.

Gene name	**Full name**	**Summary of GO terms (mainly biological process)**
CDH18	Cadherin 18	Cell-cell adhesion, cell-cell junction assembly
TSTA3	Tissue specific transplantation antigen P35B	T cell mediated cytotoxicity, nucleotide-sugar biosynthetic process (biosynthesis, usually in ER)
PRKG2	Protein kinase cGMP-dependent 2	Protein phosphorylation (regulator of intestinal secretion and bone growth)
ERN2	Endoplasmic reticulum to nucleus signaling 2	Regulation transcription, mRNA processing, protein phosphorylation, cell cycle arrest, intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, apoptotic chromosome condensation
POMC	Proopiomelanocortin	Signal transduction, regulation transcription, generation of precursor metabolites and energy, glucose homeostasis, regulation of appetite, regulation of blood pressure, regulation of glycogen metabolic process, pigmentation, regulation inflammation
LAMC3	Laminin subunit gamma 3	Cell morphogenesis, cell differentiation, visual perception, astrocyte development, retina development in camera-type eye
LOC107213704	Complement C1q subcomponent subunit C-like	Negative regulation of granulocyte differentiation, negative regulation of macrophage differentiation, synapse pruning
PROM2	Prominin 2	Cell projection organization, protein phosphorylation, regulation of GTPase activity (signal transduction)
TARS2	Threonyl-tRNA synthetase 2	tRNA aminoacylation for protein translation (protein synthesis)
PLXNA2	Plexin A2	Nervous system development, axon guidance, invasive growth and cell migration, signal transduction
LOC107198385	Ketosamine-3-kinase-like	Post-translational protein modification (phosphorylation)
ATF6	Activating transcription factor 6	Eye development, regulation transcription, endoplasmic reticulum unfolded protein response, cell apoptosis
PREB	Prolactin regulatory element binding	Protein exit from endoplasmic reticulum
LOC107198895	Rap guanine nucleotide exchange factor 3-like	Signal transduction
SCRN2	Secernin 2	Proteolysis
LOC107199222	Zinc finger protein 664-like	-
WDR83(OS)	WD repeat domain 83 (opposite strand)	Phosphorylation, MAPK cascade (cell signaling), (m)RNA splicing, mRNA processing
LOC107199333	Plectin-like	-

- *Function could not be verified*

Supplementary Table 10 | Enriched GO terms with significant FDR q-values for all the genes covered by the Control1 pool and the Control2 pool. Enrichment (N, B, n, b) is defined as (b/n) / (B/N). N is the total number of genes, B is the total number of genes associated with a specific GO term, n is the number of genes in the top of the input list, b - is the number of genes in the intersection.

Gene ontology	GO Term	Description	P-value	FDR q-value	Enrichment	N	B	n	b
Biological process	GO:0048856	anatomical structure development	1.35 × 10-12	1.95 × 10-8	1.47	11182	2437	919	294
Biological process	GO:0032502	developmental process	1.01 × 10-9	7.28 × 10-6	1.30	11182	3495	999	406
Biological process	GO:0048731	system development	1.06 × 10-8	5.09 × 10-5	1.87	11182	539	1052	95
Biological process	GO:0048513	animal organ development	1.54 × 10-6	3.69 × 10-3	1.57	11182	992	904	126
Biological process	GO:0007155	cell adhesion	1.09 × 10-6	3.93 × 10-3	2.21	11182	581	436	50
Biological process	GO:0022610	biological adhesion	1.39 × 10-6	4.01 × 10-3	2.19	11182	585	436	50
Biological process	GO:0030155	regulation of cell adhesion	1.96 × 10-6	4.04 × 10-3	1.75	11182	474	1132	84
Biological process	GO:0050772	positive regulation of axonogenesis	2.94 × 10-6	5.3 × 10-3	3.84	11182	73	758	19
Biological process	GO:0048869	cellular developmental process	3.89 × 10-6	6.23 × 10-3	1.34	11182	1991	1034	246
Biological process	GO:2000026	regulation of multicellular organismal development	4.84 × 10-6	6.97 × 10-3	1.39	11182	1525	1032	196
Biological process	GO:0050793	regulation of developmental process	6.89 × 10-6	9.03 × 10-3	1.32	11182	1902	1120	252
Biological process	GO:0010646	regulation of cell communication	8.43 × 10-6	9.35 × 10-3	1.27	11182	2509	1133	322
Biological process	GO:0045595	regulation of cell differentiation	9.34 × 10-6	9.61 × 10-3	1.41	11182	1348	1056	179
Biological process	GO:0051094	positive regulation of developmental process	1.02 × 10-5	9.78 × 10-3	1.47	11182	1024	1078	145
Biological process	GO:0023051	regulation of signaling	8.2 × 10-6	9.85 × 10-3	1.27	11182	2535	1133	325
Biological process	GO:0051241	negative regulation of multicellular organismal process	1.12 × 10-5	1.01 × 10-2	1.51	11182	836	1104	125
Biological process	GO:0035066	positive regulation of histone acetylation	1.97 × 10-5	1.58 × 10-2	10.16	11182	21	367	7
Biological process	GO:0048646	anatomical structure formation involved in morphogenesis	2.15 × 10-5	1.63 × 10-2	1.64	11182	567	1046	87
Biological process	GO:1901990	regulation of mitotic cell cycle phase transition	1.94 × 10-5	1.65 × 10-2	19.17	11182	324	9	5
Biological process	GO:1901987	regulation of cell cycle phase transition	2.58 × 10-5	1.86 × 10-2	18.01	11182	345	9	5
Biological process	GO:0050808	synapse organization	3.73 × 10-5	2.56 × 10-2	2.62	11182	143	745	25
Biological process	GO:0098609	cell-cell adhesion	3.94 × 10-5	2.58 × 10-2	2.01	11182	309	811	45
Biological process	GO:0009966	regulation of signal transduction	4.3 × 10-5	2.58 × 10-2	1.27	11182	2228	1133	286
Biological process	GO:0050770	regulation of axonogenesis	4.23 × 10-5	2.65 × 10-2	2.38	11182	149	913	29
Biological process	GO:0045597	positive regulation of cell differentiation	5.02 × 10-5	2.89 × 10-2	1.52	11182	744	1056	107
Biological process	GO:2000758	positive regulation of peptidyl-lysine acetylation	6.02 × 10-5	3.21 × 10-2	8.89	11182	24	367	7

Biological process	GO:0010557	positive regulation of macromolecule biosynthetic process	5.95 × 10-5	3.3 × 10-2	1.36	11182	1346	1104	181
Biological process	GO:0051239	regulation of multicellular organismal process	7.56 × 10-5	3.4 × 10-2	1.26	11182	2247	1104	280
Biological process	GO:0048568	embryonic organ development	7.92 × 10-5	3.46 × 10-2	2.69	11182	115	868	24
Biological process	GO:0022603	regulation of anatomical structure morphogenesis	7.52 × 10-5	3.49 × 10-2	1.48	11182	836	1046	116
Biological process	GO:0010770	positive regulation of cell morphogenesis involved in differentiation	7.47 × 10-5	3.59 × 10-2	2.72	11182	130	758	24
Biological process	GO:0007156	homophilic cell adhesion via plasma membrane adhesion molecules	7.43 × 10-5	3.69 × 10-2	3.12	11182	84	811	19
Biological process	GO:0009653	anatomical structure morphogenesis	7.42 × 10-5	3.82 × 10-2	1.41	11182	1127	1025	146
Biological process	GO:0045785	positive regulation of cell adhesion	9.21 × 10-5	3.9 × 10-2	1.86	11182	267	1104	49
Biological process	GO:0045944	positive regulation of transcription by RNA polymerase II	1.01 × 10-4	4.16 × 10-2	1.47	11182	886	1025	119
Biological process	GO:0010628	positive regulation of gene expression	1.14 × 10-4	4.22 × 10-2	1.33	11182	1460	1116	194
Biological process	GO:1902218	regulation of intrinsic apoptotic signaling pathway in response to osmotic stress	1.1 × 10-4	4.3 × 10-2	152.14	11182	3	49	2
Biological process	GO:0021615	glossopharyngeal nerve morphogenesis	1.14 × 10-4	4.31 × 10-2	12.56	11182	4	890	4
Biological process	GO:0007399	nervous system development	1.2 × 10-4	4.31 × 10-2	1.95	11182	212	1108	41
Biological process	GO:0003006	developmental process involved in reproduction	1.23 × 10-4	4.32 × 10-2	1.74	11182	446	881	61
Biological process	GO:0010769	regulation of cell morphogenesis involved in differentiation	1.28 × 10-4	4.4 × 10-2	1.91	11182	247	1041	44
Biological process	GO:1902219	negative regulation of intrinsic apoptotic signaling pathway in response to osmotic stress	1.1 × 10-4	4.42 × 10-2	152.14	11182	3	49	2
Biological process	GO:0035065	regulation of histone acetylation	1.39 × 10-4	4.67 × 10-2	5.85	11182	40	430	9
Biological process	GO:0007346	regulation of mitotic cell cycle	1.51 × 10-4	4.73 × 10-2	12.73	11182	488	9	5
Biological process	GO:1903508	positive regulation of nucleic acid-templated transcription	1.46 × 10-4	4.77 × 10-2	1.37	11182	1173	1104	159
Biological process	GO:0045746	negative regulation of Notch signaling pathway	1.49 × 10-4	4.78 × 10-2	3.90	11182	31	1111	12
Biological process	GO:1902680	positive regulation of RNA biosynthetic process	1.56 × 10-4	4.78 × 10-2	1.37	11182	1174	1104	159
Cellular component	GO:0044459	plasma membrane part	1.37 × 10-6	2.55 × 10-3	1.37	11182	1731	1077	229
Cellular component	GO:0005886	plasma membrane	1.07 × 10-5	6.63 × 10-3	1.25	11182	2645	1132	336
Cellular component	GO:0005891	voltage-gated calcium channel complex	9.6 × 10-6	8.95 × 10-3	5.61	11182	27	812	11
Cellular component	GO:0060077	inhibitory synapse	9.12 × 10-5	2.43 × 10-2	7.36	11182	15	709	7
Cellular component	GO:0031226	intrinsic component of plasma membrane	8.59 × 10-5	2.67 × 10-2	1.46	11182	872	1070	122

Cellular component	GO:0000785	chromatin	8.17 × 10-5	3.05 × 10-2	1.51	11182	863	929	108
Cellular component	GO:0030424	axon	7.72 × 10-5	3.6 × 10-2	3.26	11182	242	255	18
Cellular component	GO:0120044	stereocilium base	1.64 × 10-4	3.81 × 10-2	159.74	11182	4	35	2
Molecular function	GO:0001067	regulatory region nucleic acid binding	5.84 × 10-6	2.19 × 10-3	1.57	11182	800	1014	114
Molecular function	GO:1990837	sequence-specific double-stranded DNA binding	5.73 × 10-6	2.37 × 10-3	1.56	11182	853	1003	119
Molecular function	GO:0044212	transcription regulatory region DNA binding	5.51 × 10-6	2.53 × 10-3	1.57	11182	799	1014	114
Molecular function	GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	1.25 × 10-6	2.58 × 10-3	1.70	11182	643	995	97
Molecular function	GO:0000976	transcription regulatory region sequence-specific DNA binding	5.19 × 10-6	2.68 × 10-3	1.58	11182	798	1014	114
Molecular function	GO:0004714	transmembrane receptor protein tyrosine kinase activity	4.6 × 10-6	2.71 × 10-3	3.56	11182	53	1125	19
Molecular function	GO:0043565	sequence-specific DNA binding	8.51 × 10-6	2.93 × 10-3	1.52	11182	923	1003	126
Molecular function	GO:0019199	transmembrane receptor protein kinase activity	2.2 × 10-6	3.02 × 10-3	3.25	11182	70	1132	23
Molecular function	GO:0001012	RNA polymerase II regulatory region DNA binding	4.54 × 10-6	3.12 × 10-3	1.61	11182	731	995	105
Molecular function	GO:0000987	proximal promoter sequence-specific DNA binding	8.73 × 10-7	3.6 × 10-3	1.70	11182	655	995	99
Molecular function	GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	4.54 × 10-6	3.75 × 10-3	1.61	11182	731	995	105
Molecular function	GO:0140110	transcription regulator activity	3.96 × 10-6	4.09 × 10-3	1.51	11182	1110	929	139
Molecular function	GO:0003690	double-stranded DNA binding	2.33 × 10-5	7.41 × 10-3	1.50	11182	914	1003	123
Molecular function	GO:0043121	neurotrophin binding	3.35 × 10-5	9.89 × 10-3	14.50	11182	8	482	5
Molecular function	GO:0000981	DNA-binding transcription factor activity, RNA polymerase II-specific	4.5 × 10-5	1.24 × 10-2	1.56	11182	709	990	98
Molecular function	GO:0004137	deoxycytidine kinase activity	8.08 × 10-5	2.08 × 10-2	110.71	11182	2	101	2
Molecular function	GO:0003700	DNA-binding transcription factor activity	1.14 × 10-4	2.77 × 10-2	1.52	11182	734	990	99
Molecular function	GO:0005003	ephrin receptor activity	1.62 × 10-4	3.52 × 10-2	13.00	11182	17	253	5
Molecular function	GO:0008013	beta-catenin binding	1.61 × 10-4	3.7 × 10-2	3.52	11182	72	662	15

Supplementary Table 11 | Enriched GO terms with significant FDR q-values for all the genes covered by the Reduced pool and the Enlarged pool. Enrichment (N, B, n, b) is defined as (b/n) / (B/N). N is the total number of genes, B is the total number of genes associated with a specific GO term, n is the number of genes in the top of the input list, b - is the number of genes in the intersection.

Ontology	GO Term	Description	P-value	FDR q-value	Enrichment	N	B	n	b
Biological process	GO:0032502	developmental process	1.14 × 10-9	1.64 × 10-5	1.29	11210	3508	1027	416
Biological process	GO:0009653	anatomical structure morphogenesis	9.79 × 10-9	4.71 × 10-5	1.55	11210	1131	1134	177
Biological process	GO:0051239	regulation of multicellular organismal process	9.32 × 10-9	6.72 × 10-5	1.38	11210	2257	1021	284
Biological process	GO:0045595	regulation of cell differentiation	3.32 × 10-8	9.59 × 10-5	1.48	11210	1352	1135	202
Biological process	GO:0048856	anatomical structure development	3.03 × 10-8	1.09 × 10-4	1.35	11210	2448	1025	302
Biological process	GO:0048731	system development	3.09 × 10-7	7.44 × 10-4	1.90	11210	538	846	77
Biological process	GO:0051254	positive regulation of RNA metabolic process	6.48 × 10-7	1.34 × 10-3	1.46	11210	1241	1129	182
Biological process	GO:0045944	positive regulation of transcription by RNA polymerase II	7.79 × 10-7	1.41 × 10-3	1.55	11210	889	1113	137
Biological process	GO:0048869	cellular developmental process	1.53 × 10-6	2.46 × 10-3	1.33	11210	1998	1129	268
Biological process	GO:1902680	positive regulation of RNA biosynthetic process	3.55 × 10-6	3.01 × 10-3	1.44	11210	1175	1117	169
Biological process	GO:0010628	positive regulation of gene expression	2.11 × 10-6	3.04 × 10-3	1.40	11210	1465	1129	206
Biological process	GO:2000026	regulation of multicellular organismal development	2.56 × 10-6	3.08 × 10-3	1.38	11210	1530	1135	214
Biological process	GO:0044057	regulation of system process	2.37 × 10-6	3.11 × 10-3	1.80	11210	423	1120	76
Biological process	GO:0065007	biological regulation	3.47 × 10-6	3.13 × 10-3	1.10	11210	7555	1076	799
Biological process	GO:0032501	multicellular organismal process	2.86 × 10-6	3.17 × 10-3	1.34	11210	2259	921	248
Biological process	GO:0045893	positive regulation of transcription, DNA-templated	3.4 × 10-6	3.27 × 10-3	1.46	11210	1123	1117	163
Biological process	GO:1903508	positive regulation of nucleic acid-templated transcription	3.36 × 10-6	3.47 × 10-3	1.44	11210	1174	1117	169
Biological process	GO:0050793	regulation of developmental process	1.08 × 10-5	8.2 × 10-3	1.31	11210	1909	1135	254
Biological process	GO:0048513	animal organ development	1.04 × 10-5	8.34 × 10-3	1.46	11210	997	1130	147
Biological process	GO:0050794	regulation of cellular process	1.3 × 10-5	9.34 × 10-3	1.11	11210	6736	1135	758
Biological process	GO:0031328	positive regulation of cellular biosynthetic process	1.57 × 10-5	1.08 × 10-2	1.38	11210	1419	1096	191
Biological process	GO:0045664	regulation of neuron differentiation	1.92 × 10-5	1.26 × 10-2	1.66	11210	535	1061	84
Biological process	GO:0006357	regulation of transcription by RNA polymerase II	2.1 × 10-5	1.27 × 10-2	1.34	11210	1640	1096	215
Biological process	GO:0009891	positive regulation of biosynthetic process	2.08 × 10-5	1.3 × 10-2	1.37	11210	1434	1096	192

Biological process	GO:0050789	regulation of biological process	2.25 × 10-5	1.3 × 10-2	1.11	11210	7151	1025	723
Biological process	GO:0009887	animal organ morphogenesis	2.51 × 10-5	1.39 × 10-2	1.85	11210	417	858	59
Biological process	GO:0010557	positive regulation of macromolecule biosynthetic process	3.13 × 10-5	1.67 × 10-2	1.37	11210	1347	1117	184
Biological process	GO:0045935	positive regulation of nucleobase-containing compound metabolic process	3.42 × 10-5	1.76 × 10-2	1.36	11210	1394	1129	191
Biological process	GO:0048646	anatomical structure formation involved in morphogenesis	3.97 × 10-5	1.91 × 10-2	1.78	11210	567	711	64
Biological process	GO:0030154	cell differentiation	3.86 × 10-5	1.92 × 10-2	1.36	11210	1356	1134	187
Biological process	GO:0048167	regulation of synaptic plasticity	5.19 × 10-5	2.41 × 10-2	3.27	11210	145	426	18
Biological process	GO:0051240	positive regulation of multicellular organismal process	8.68 × 10-5	3.91 × 10-2	1.39	11210	1261	1020	159
Biological process	GO:0048522	positive regulation of cellular process	9.08 × 10-5	3.97 × 10-2	1.19	11210	3756	1023	408
Biological process	GO:0045927	positive regulation of growth	9.51 × 10-5	4.04 × 10-2	2.08	11210	190	1020	36
Biological process	GO:0032879	regulation of localization	1.01 × 10-4	4.06 × 10-2	1.28	11210	1956	1119	250
Biological process	GO:1901385	regulation of voltage-gated calcium channel activity	1 × 10-4	4.12 × 10-2	8.43	11210	29	321	7
Biological process	GO:0045597	positive regulation of cell differentiation	1.06 × 10-4	4.12 × 10-2	1.48	11210	747	1134	112
Biological process	GO:0090257	regulation of muscle system process	1.22 × 10-4	4.63 × 10-2	2.09	11210	163	1120	34
Biological process	GO:0050767	regulation of neurogenesis	1.33 × 10-4	4.92 × 10-2	1.53	11210	655	1061	95
Molecular function	GO:0000981	DNA-binding transcription factor activity, RNA polymerase II-specific	1.32 × 10-5	1.09 × 10-2	1.55	11210	709	1129	111
Molecular function	GO:0000987	proximal promoter sequence-specific DNA binding	1.12 × 10-5	1.16 × 10-2	1.59	11210	653	1113	103
Molecular function	GO:0001012	RNA polymerase II regulatory region DNA binding	9.85 × 10-6	1.36 × 10-2	1.55	11210	729	1129	114
Molecular function	GO:0003700	DNA-binding transcription factor activity	2.32 × 10-5	1.6 × 10-2	1.53	11210	734	1129	113
Molecular function	GO:0001067	regulatory region nucleic acid binding	4.08 × 10-5	1.87 × 10-2	1.49	11210	799	1129	120
Molecular function	GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding	4.61 × 10-6	1.9 × 10-2	1.62	11210	641	1113	103
Molecular function	GO:0044212	transcription regulatory region DNA binding	3.74 × 10-5	1.93 × 10-2	1.49	11210	798	1129	120
Molecular function	GO:0140110	transcription regulator activity	4.71 × 10-5	1.95 × 10-2	1.40	11210	1110	1129	157
Molecular function	GO:0000977	RNA polymerase II regulatory region sequence-specific DNA binding	9.85 × 10-6	2.03 × 10-2	1.55	11210	729	1129	114

Molecular function	GO:0000976	transcription regulatory region sequence-specific DNA binding	3.57 × 10-5	2.11 × 10-2	1.49	11210	797	1129	120
Molecular function	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	1.04 × 10-4	3.89 × 10-2	1.77	11210	314	1129	56
Cellular component	GO:0034703	cation channel complex	2.74 × 10-5	2.55 × 10-2	2.29	11210	161	1005	33

Supplementary Table 12 | Location, chromosome number or unplaced scaffold, p-value, ∆ of % methylation level, region and gene name associated with DMS between the Enlarged pool and the Reduced pool with minimally 10% differential methylation.

Location DMS	**Chrom./scaf.**	**P-value**	**∆ Meth.**	**Region**	**Gene**
751892	chr1	8.00×10^{-10}	-22%	downstream	*LOC107199779*
40214348	chr1	1.41×10^{-7}	21%	intron	*UGGT2*
68603889	chr1	1.56×10^{-8}	-21%	TSS	*MTIF3*
74232041	chr1	8.27×10^{-8}	-15%		-
102335015	chr1	5.79×10^{-8}	10%	intron	*LOC107212328*
103471071	chr1	4.25×10^{-9}	19%	TSS	*LOC107210103*
65874327	chr1A	1.01×10^{-7}	16%	intron	*PIK3C2G*
24814973	chr2	8.73×10^{-8}	-14%	downstream	*DLX5*
				upstream	*LOC107200304*
24815638	chr2	1.78×10^{-11}	-24%	downstream	*DLX5*
58234244	chr2	3.40×10^{-9}	21%		-
75729799	chr2	5.62×10^{-10}	-27%	intron	*CDH18*
149822731	chr2	4.28×10^{-9}	34%	TSS	*TSTA3*
11777958	chr3	1.55×10^{-9}	21%	exon	*LOC107201964*
30236804	chr3	1.78×10^{-8}	18%		-
30782889	chr3	4.69×10^{-12}	-13%	upstream	*NFKBIE*
57049580	chr3	1.41×10^{-10}	-11%	TSS	*AHI1*
57049624	chr3	3.25×10^{-12}	-11%	TSS	*AHI1*
1948598	chr4	8.95×10^{-8}	23%	exon	*ADAMTS3*
13870784	chr4	1.04×10^{-13}	72%	intron	*PRKG2*
67723291	chr4	4.92×10^{-12}	-24%	upstream	*LOC107202670*
564313	chr4A	4.99×10^{-17}	-26%		-
7481495	chr4A	1.40×10^{-8}	12%	intron	*HS6ST2*
10966189	chr4A	8.75×10^{-8}	-15%	intron	*TENM1*
12090213	chr4A	3.92×10^{-9}	11%	upstream	*NXT2*
60889445	chr5	4.43×10^{-13}	22%		-
61769830	chr5	1.74×10^{-8}	-16%		-
4132908	chr6	1.92×10^{-10}	20%	intron	*JMJD1C*
32813644	chr6	7.27×10^{-9}	27%		-
3866145	chr9	7.51×10^{-10}	-20%	downstream	*FOXL2*
5614366	chr9	4.59×10^{-8}	18%	exon	*C9H3orf70*
19323964	chr10	8.13×10^{-11}	17%		-
19467110	chr10	2.71×10^{-8}	-21%	promoter	*UACA*
4107071	chr13	6.37×10^{-8}	17%		-
1441126	chr14	6.32×10^{-8}	-31%	upstream	*PPL*
15624191	chr14	1.86×10^{-9}	-25%	exon	*ERN2*
15631118	chr14	2.16×10^{-8}	-19%	promoter	*ERN2*
3434986	chr15	7.46×10^{-8}	20%	intron	*CUX2*
12084377	chr15	5.43×10^{-12}	11%	promoter	*RFC5*
13816573	chr15	1.10×10^{-9}	-30%	exon	*POMC*

2340658	chr17	4.27×10^{-8}	20%	promoter	*UBAC1*
4729995	chr17	1.42×10^{-14}	-26%	intron	*LAMC3*
4730000	chr17	7.06×10^{-11}	-26%	intron	*LAMC3*
4730098	chr17	1.67×10^{-9}	-19%	exon	*LAMC3*
4730148	chr17	1.42×10^{-17}	-26%	exon	*LAMC3*
110	chr18	1.12×10^{-12}	10%	upstream	*CSNK1D*
2363952	chr18	1.66×10^{-8}	29%	downstream	*LOC107212597*
514697	chr20	2.08×10^{-9}	19%	upstream	*NDRG3*
1513470	chr21	4.52×10^{-8}	21%	exon	*CTRC*
1797839	chr21	1.17×10^{-12}	-45%	promoter	*LOC107213704*
729950	chr22	1.73×10^{-10}	-24%	promoter	*PROM2*
730012	chr22	1.36×10^{-13}	70%	promoter	*PROM2*
3601967	chr22	1.08×10^{-7}	18%	intron	*ADGRA2*
162665	chr23	7.49×10^{-10}	14%	promoter	*AIM1L*
2214814	chr23	9.05×10^{-10}	23%	intron	*COL16A1*
5854480	chr23	6.54×10^{-10}	-27%	-	-
6296431	chr23	5.72×10^{-8}	-29%	-	-
6427084	chr23	3.46×10^{-12}	37%	downstream	*LOC107214194*
597551	chr25LG2	1.89×10^{-8}	-25%	intron	*TARS2*
5506639	chr26	2.27×10^{-8}	26%	intron	*PLXNA2*
70917608	chrZ	4.27×10^{-8}	26%	TSS	*LOC107198385*
476	scaffold	1.23×10^{-10}	25%	-	-
506	scaffold	4.22×10^{-8}	-26%	-	-
583	scaffold	1.54×10^{-7}	12%	-	-
913	scaffold	4.13×10^{-9}	41%	-	-
1469	scaffold	3.46×10^{-9}	23%	-	-
1719	scaffold	1.70×10^{-8}	-31%	-	-
1772	scaffold	6.52×10^{-8}	28%	promoter	*LOC107199333*
4319	scaffold	1.70×10^{-8}	57,5%	-	-
4405	scaffold	1.36×10^{-7}	22%	intron	*LOC107199430*
11100	scaffold	4.62×10^{-8}	41%	-	-
11884	scaffold	1.49×10^{-10}	-53%	promoter	*WDR83OS*
17628	scaffold	7.56×10^{-12}	15%	-	-
19200	scaffold	4.18×10^{-8}	29%	TSS	*LOC107199222*
43671	scaffold	6.12×10^{-8}	16%	intron	*LOC107199006*
50776	scaffold	3.64×10^{-8}	17%	promoter	*PREB*
50860	scaffold	1.82×10^{-14}	-34%	promoter	*PREB*
51297	scaffold	8.51×10^{-9}	19%	promoter	*PREB*
66988	scaffold	6.78×10^{-10}	-26%	intron	*SCRN2*
73459	scaffold	6.10×10^{-10}	25%	intron	*LOC107198895*
246819	scaffold	1.77×10^{-7}	-29%	promoter	*ATF6*
612607	scaffold	2.54×10^{-8}	-19%	TSS	*ANKRD33*
860948	scaffold	3.13×10^{-12}	20%	upstream	*HSPA9*

- *Not annotated*

Supplementary Table 13 | Location, chromosome number or unplaced scaffold, p-value, Δ of % methylation level, region and gene name associated with DMS between pool Control1 and pool Control2 with minimally 10% differential methylation.

Location DMS	**Chrom./scaf.**	**P-value**	**Δ Meth.**	**Region**	**Gene**
113634610	chr1	4.24×10^{-08}	71%	-	-
57317668	chr1A	1.15×10^{-08}	21%	downst	*BHLHE41*
80410210	chr2	1.11×10^{-07}	19%	-	-
9668340	chr4A	1.61×10^{-10}	47%	-	-
54944183	chr5	1.68×10^{-08}	26%	promoter	*HSPA2*
60057909	chr5	1.88×10^{-07}	77%	downst	*ARF6*
17727814	chr7	2.12×10^{-07}	28%	promoter	*METTL8*
37135105	chr7	1.87×10^{-09}	62%	downst	*CYTIP*
4427330	chr10	2.30×10^{-08}	32%	intron	*RORA*
14791064	chr14	1.46×10^{-07}	71%	exon	*LOC107211364*
2212920	chr17	5.56×10^{-08}	80%	intron	*KCNT1*
919719	chr18	2.57×10^{-08}	85%	downst	*CHMP6*
				upst	*BAIAP2*
7556239	chr19	5.88×10^{-08}	71%	upst	*TBX2*
1171186	chr20	1.57×10^{-07}	-75%	-	-
6759952	chr20	8.15×10^{-08}	28%	TSS	*LOC107213098*
200856	chr22	9.46×10^{-20}	34%	intron	*TGOLN2*
11977179	chrZ	5.05×10^{-11}	25%	TSS	*LOC107198170*
68877019	chrZ	2.42×10^{-10}	37%	intron	*APC*
22299	scaffold	1.16×10^{-07}	22%	-	-
175111	scaffold	7.77×10^{-08}	33%	intron	*ATF7*

- *Not annotated*

Supplementary Table 14 | Number of DMS between Control1 pool and Control2 pool and between the Enlarged pool and the Reduced pool (minimally 10% differential methylation), number of DMS found in one comparison that is also found in het other comparison and number and percentage of DMS found in one comparison that is covered in the other comparison.

Comparison	**# DMS**	**# Overlapping DMS in other comparison**	**# Covered in other comparison**	**Percentage covered in other comparison**
Control1 vs. Control2	20	0	14	70%
Reduced vs. Enlarged	82	0	74	93%

Supplementary Table 15 | Gene name, full name and biological functions associated with DMS between the Control1 pool and the Control2 pool with minimally 10% differential methylation.

Gene name	**Full name**	**Summary of GO terms (mainly biological process)**
HSPA2	Heat shock protein family A (Hsp70) member 2	Protein refolding, spermatogenesis
METTL8	Methyltransferase like 8	Skeletal muscle tissue development, fat cell differentiation, histone acetylation, mRNA methylation
LOC107198170	Oncostatin-M-specific receptor subunit beta-like	Cell population proliferation, inflammatory response
LOC107213098	Zinc finger SWIM domain-containing protein 1	Zinc and metal ion binding
LOC107211364	Testis-expressed sequence 2 protein-like	-
ATF7	Activating transcription factor 7	Regulation transcription
APC	APC regulator of WNT signaling pathway	Cell proliferation (negative), tissue formation, cell migration
TGOLN2	Trans-golgi network protein 2	Post-translational protein modification, cellular protein metabolic process, membrane organization (exocytic vesicle formation)
KCNT1	Potassium sodium-activated channel subfamily T member 1	Potassium ion transport (neuronal excitability)
RORA	RAR related orphan receptor A	Transcriptional regulation, maintenance circadian rhythm, development of cerebellum, fat cell differentiation, muscle cell development, immune response
BHLHE41	basic helix-loop-helix family member e41	Anterior/posterior pattern specification, circadian regulation gene expression, negative regulation myotube differentiation, regulation neurogenesis, regulation transcription
ARF6	ADP ribosylation factor 6	Organization actin cytoskeleton, endocytic recycling, establishment epithelial cell polarity, hepatocyte and myeloid cell apoptotic process, intracellular protein transport, focal adhesion disassembly, keratinocyte migration, protein secretion, dendritic spine and liver development, ruffle and filopodium assembly, Rac protein signal transduction
CYTIP	cytohesin 1 interacting protein	Regulation of cell adhesion
CHMP6	charged multivesicular body protein 6	Late endosome to vacuole transport via multivesicular body sorting pathway, midbody abscission, mitotic metaphase plate congression, negative regulation epidermal growth factor-activated receptor

		activity, nucleus organization, protein transport, protein catabolic process, vesicle budding from membrane, viral budding via host ESCRT complex
BAIAP2	Brain-specific angiogenesis inhibitor 1-associated protein 2	Actin crosslink formation, actin filament bundle assembly, dendrite development, plasma membrane organization, actin cytoskeleton reorganization, actin filament polymerization, cell shape, synaptic plasticity, response to bacterium
TBX2	T-box transcription factor 2	Aorta morphogenesis, atrioventricular canal and roof of mouth and pharynx cardiac muscle tissue development, cell fate specification, cellular senescence, developmental growth involved in morphogenesis, embryonic camera-type eye morphogenesis, embryonic digit morphogenesis, endocardial cushion morphogenesis, muscle cell fate determination, negative regulation heart looping and cardiac chamber formation, transcription by RNA polymerase II, Notch signaling pathway, outflow tract septum morphogenesis, cardiac muscle cell proliferation, smooth muscle cell differentiation, ureteric peristalsis

- *Uncharacterized or function could not be verified*

Supplementary Table 16 | Gene name, full name and biological functions associated with DMS between the Reduced pool and Enlarged pool with minimally 10% differential methylation.

Gene name	Full name	Summary of GO terms (mainly biological process)
CDH18	Cadherin 18	Cell-cell adhesion, cell-cell junction assembly
TSTA3	Tissue specific transplantation antigen P35B	T cell mediated cytotoxicity, nucleotide-sugar biosynthetic process (biosynthesis, usually in ER)
PRKG2	Protein kinase cGMP-dependent 2	Protein phosphorylation (regulator intestinal secretion and bone growth)
ERN2	Endoplasmic reticulum to nucleus signaling 2	Regulation transcription, mRNA processing, protein phosphorylation, cell cycle arrest, intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress, apoptotic chromosome condensation
POMC	Proopiomelanocortin	Signal transduction, regulation transcription, generation precursor metabolites and energy, glucose homeostasis, regulation appetite, regulation blood pressure, regulation glycogen metabolic process, pigmentation, regulation inflammation
LAMC3	Laminin subunit gamma 3	Cell morphogenesis, cell differentiation, visual perception, astrocyte development, retina development in camera-type eye
LOC107213704	Complement C1q subcomponent subunit C-like	Negative regulation granulocyte differentiation, negative regulation macrophage differentiation, synapse pruning
PROM2	Prominin 2	Cell projection organization, protein phosphorylation, regulation GTPase activity (signal transduction)
TARS2	Threonyl-tRNA synthetase 2	tRNA aminoacylation for protein translation (protein synthesis)
PLXNA2	Plexin A2	Nervous system development, axon guidance, invasive growth and cell migration, signal transduction
LOC107198385	Ketosamine-3-kinase-like	Post-translational protein modification (phosphorylation)
ATF6	Activating transcription factor 6	Eye development, regulation transcription, endoplasmic reticulum unfolded protein response, cell apoptosis
PREB	Prolactin regulatory element binding	Protein exit from endoplasmic reticulum
LOC107198895	Rap guanine nucleotide exchange factor 3-like	Signal transduction
SCRN2	Secernin 2	Proteolysis
LOC107199222	Zinc finger protein 664-like	-
WDR83(OS)	WD repeat domain 83 (opposite strand)	Phosphorylation, MAPK cascade (cell signaling), (m)RNA splicing, mRNA processing
LOC107199333	Plectin-like	-
LOC107199779	-	-
UGGT2	UDP-glucose glycoprotein glucosyltransferase 2	ER-associated misfolded protein catabolic process, protein N-linked glycosylation via asparagine

MTIF3	mitochondrial translational initiation factor 3	Mitochondrial translational initiation, ribosome disassembly
LOC107212328	nuclear mitotic apparatus protein 1-like	Anastral spindle assembly, astral microtubule organization, establishment mitotic spindle orientation, Golgi ribbon and microtubule bundle and microtubule organizing center formation, endosome to Golgi and intracellular transport, BMP signaling pathway, chromosome separation, hair follicle development, keratinocyte differentiation, microtubule polymerization, mitotic spindle elongation, protein localization to cell cortex, protein localization to spindle pole body, spindle assembly, protein localization to Golgi apparatus, protein targeting to lysosome, metaphase plate congression, protein exit from endoplasmic reticulum
LOC107210103	-	-
PIK3C2G	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma	Cell migration, phosphatidylinositol 3-kinase signaling, phosphatidylinositol-3-phosphate biosynthetic process, phosphatidylinositol-mediated signaling, phosphatidylinositol phosphorylation
DLX5	distal-less homeobox 5	Anatomical structure formation involved in morphogenesis, BMP signaling pathway, cell population epithelial cell proliferation, face and inner ear and embryonic limb morphogenesis, endochondral ossification, interneuron axon guidance, olfactory bulb interneuron and osteoblast and epithelial cell differentiation, olfactory pit and roof mouth development, canonical Wnt signaling pathway, transcription by RNA polymerase II
LOC107200304	-	-
LOC107201964	serine/threonine-protein kinase pim-1-like	Cellular detoxification, negative regulation apoptotic process, negative regulation DNA-binding transcription factor activity, brown fat cell differentiation, cardiac muscle cell and cardioblast and hematopoietic stem cell proliferation, transcription, DNA-templated, protein autophosphorylation, protein stabilization, transmembrane transporter activity, vitamin D receptor signaling pathway
NFKBIE	NFKB inhibitor epsilon	Cytosol, fibrillar center, nucleoplasm
AHI1	Abelson helper integration site 1	Identical protein binding
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif 3	Collagen biosynthetic process, extracellular matrix organization, vascular endothelial growth factor signaling pathway, protein processing, vascular endothelial growth factor production
LOC107202670	transcription factor COE1-like	
HS6ST2	Heparan-sulfate 6-O-sulfotransferase 2	Heparan sulfate proteoglycan biosynthetic process, enzymatic modification, limb development
TENM1	teneurin transmembrane protein 1	Heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules, neuron development, neuropeptide signaling pathway, actin filament polymerization, filopodium assembly, intracellular protein transport, MAP kinase activity, peptidyl-serine phosphorylation, transcription by RNA polymerase III
NXT2	nuclear transport factor 2 like export factor 2	mRNA transport, nucleocytoplasmic transport, protein import into nucleus

JMJD1C	jumonji domain containing 1C	Histone H3-K9 demethylation, transcription by RNA polymerase II
FOXL2	forkhead box L2	Anatomical structure morphogenesis, apoptotic DNA fragmentation, cell differentiation, embryonic eye morphogenesis, extraocular skeletal muscle and ovarian follicle and uterus development, female somatic sex determination, granulosa cell differentiation, oocyte growth, apoptotic process, follicle-stimulating hormone secretion, luteinizing hormone secretion, regulation transcription, single fertilization
C9H3orf70	chromosome 9 C3orf70 homolog	Circadian behaviour, nervous system development
UACA	uveal autoantigen with coiled-coil domains and ankyrin repeats	Apoptotic signaling pathway, negative regulation NIK/NF-kappaB signaling, positive regulation cysteine-type endopeptidase activity involved in apoptotic process
PPL	periplakin	Intermediate filament cytoskeleton organization, response to mechanical stimulus, wound healing
CUX2	cut like homeobox 2	Cellular response to organic substance, Golgi vesicle transport, negative regulation transcription by RNA polymerase II, dendritic spine morphogenesis, excitatory postsynaptic potential, gene expression, synapse assembly, short-term memory
RFC5	replication factor C subunit 5	DNA-dependent DNA replication, DNA repair, positive regulation DNA-directed DNA polymerase activity
UBAC1	UBA domain containing 1	Protein ubiquitination
CSNK1D	casein kinase 1 delta	Circadian regulation gene expression, non-motile cilium assembly, peptidyl-serine phosphorylation, (non-)canonical Wnt signaling pathway, proteasomal ubiquitin-dependent protein catabolic process, circadian rhythm, spindle assembly
LOC107212597	ATP-binding cassette sub-family A member 9-like	Lipid transport
NDRG3	NDRG family member 3	Cytoskeleton, membrane, ATP binding, non-membrane spanning protein tyrosine kinase activity, protein phosphatase binding, interleukin-4-mediated signaling pathway, intracellular signal transduction
CTRC	chymotrypsin C	Cellular calcium ion homeostasis, proteolysis
ADGRA2	adhesion G protein-coupled receptor A2	Cell surface receptor signaling pathway , central nervous system development, endothelial cell migration, negative regulation vascular endothelial growth factor signaling pathway, canonical Wnt signaling pathway, endothelial cell migration, angiogenesis, chemotaxis, establishment blood-brain barrier, sprouting angiogenesis
AIM1L	crystallin beta-gamma domain containing 2	Carbohydrate binding
COL16A1	collagen type XVI alpha 1 chain	Extracellular matrix organization
LOC107214194	lethal(3)malignant brain tumor-like protein 3	Chromatin organization, erythrocyte maturation, granulocyte differentiation, macrophage differentiation, negative regulation transcription, DNA-templated
LOC107199430	tyrosine-protein kinase JAK3-like	Adaptive immune response, B cell and erythrocyte differentiation, cytokine-mediated signaling pathway, enzyme linked receptor protein signaling pathway, growth hormone receptor signaling pathway via JAK-STAT, innate immune response, interleukin-mediated signaling pathway, intracellular signal transduction, MAPK cascade, negative regulation FasL and dendritic cell cytokine production, negative regulation

		interleukin-production and T cell activation, negative regulation T-helper 1 cell differentiation, peptidyl-tyrosine phosphorylation, T cell proliferation, protein phosphorylation, apoptotic process, receptor signaling pathway via JAK-STAT, T cell apoptotic process, response to interleukin-15, response to interleukin, T cell homeostasis, tyrosine phosphorylation STAT protein
LOC107199006	-	-
ANKRD33	ankyrin repeat domain 33	Skeletal muscle cell differentiation
HSPA9	heat shock protein family A (Hsp70) member 9	Cellular response to unfolded protein, chaperone cofactor-dependent protein refolding, iron-sulfur cluster assembly, negative regulation erythrocyte and hematopoietic stem cell differentiation, protein export from nucleus, protein refolding

- *Uncharacterized or function could not be verified*