Inventory of Supplementary Materials

SUPPLEMENTARY FIGURES

Figure S1

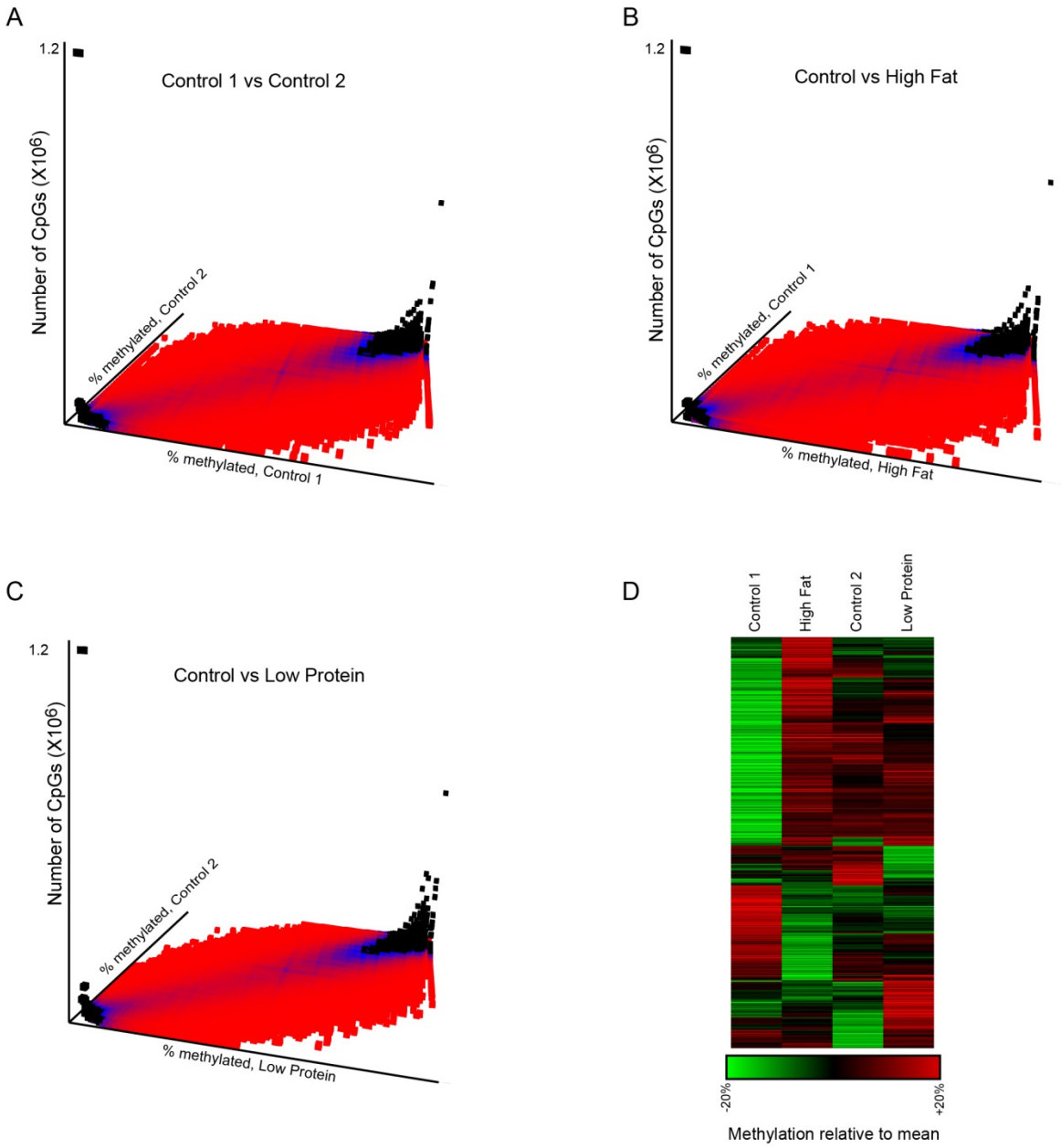

Figure S1, Related to Figure 1. Scatterplots of methylation levels between WGBS pools. (**A-C**) 3D plots showing cytosine methylation percentage for two WGBS pools (x and y axes, scale is from 0 to 100%), with z axis showing the number of individual CpGs. For each scatterplot only the 80% of genomic CpGs with the highest sequencing depth were used. Note that the vast majority of individual CpGs are clustered around (0,0) and (100,100). (**D**) Differentially-methylated regions are generally epivariable. All 300 bp windows with significant (q value < 0.05) differences between Control 1 and

High Fat, or Control 2 and Low Protein WGBS datasets, were selected. Data were zero centered, and the 2427 windows with at least one dataset exhibiting a 10% methylation difference were selected and clustered. Importantly, for the majority of windows exhibiting a difference between High Fat and Control 1, or between Low Protein and Control 2, Control 1 and Control 2 datasets also differed, indicating that these regions exhibit epivariation among Control pools.

Figure S2

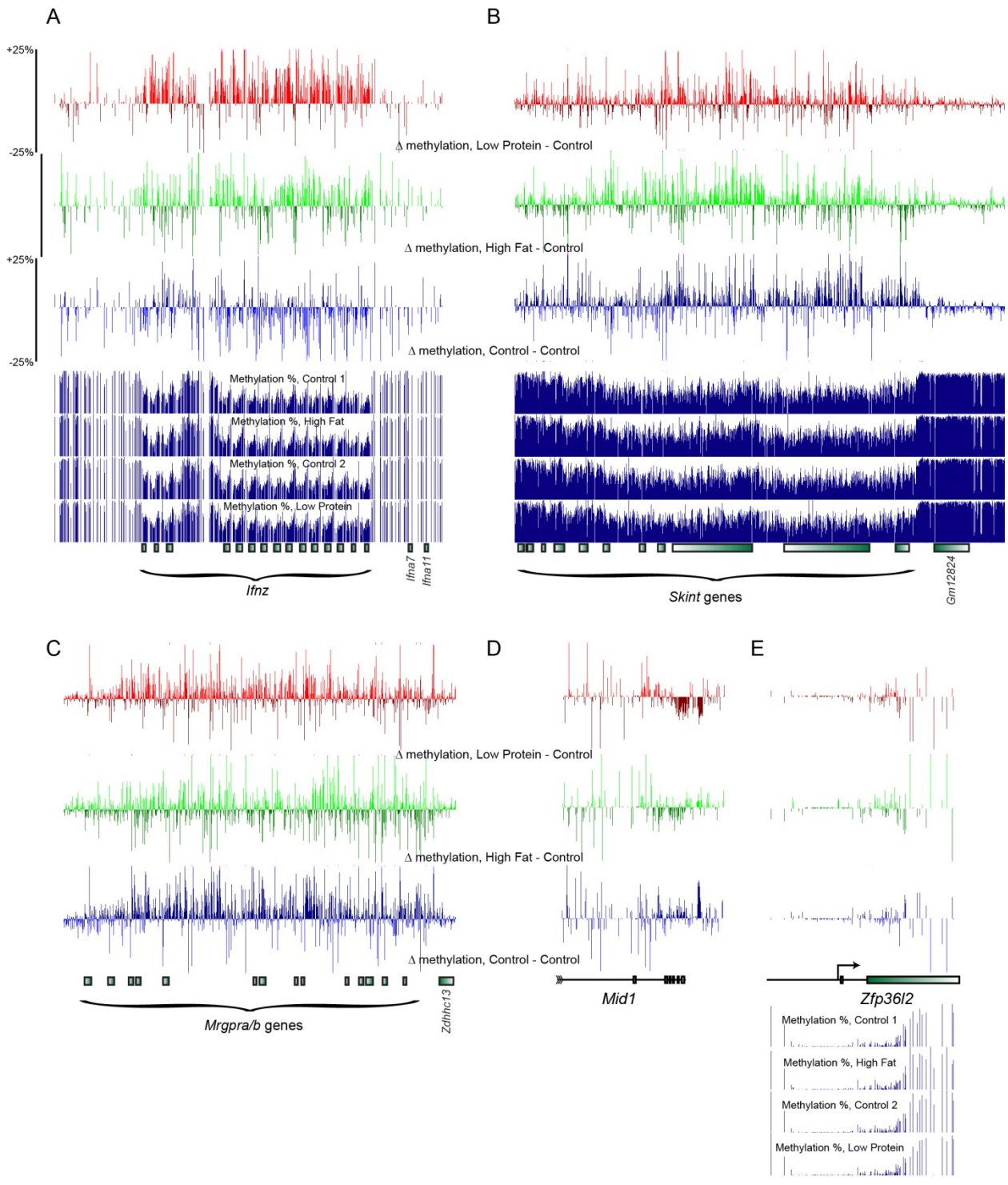

Figure S2, Related to Figure 2. Examples of diet-induced differential methylation. (**A-E**) For each locus, data are shown for Low Protein minus Control 2 (top panel, red), High Fat minus Control 1 (middle panel, green), and Control 1 minus Control 2 (bottom

panel, blue). Y axis is consistently -25% to +25%. For some loci, absolute methylation levels are also shown. Loci include multigene families *Ifnz* (**A**), *Skint* (**B**), and *Mgrpra/b* (**C**), the beginning of the X chromosome pseudoautosomal region (**D**), and an example of an epivariable CpG island shore (**E**).

Figure S3

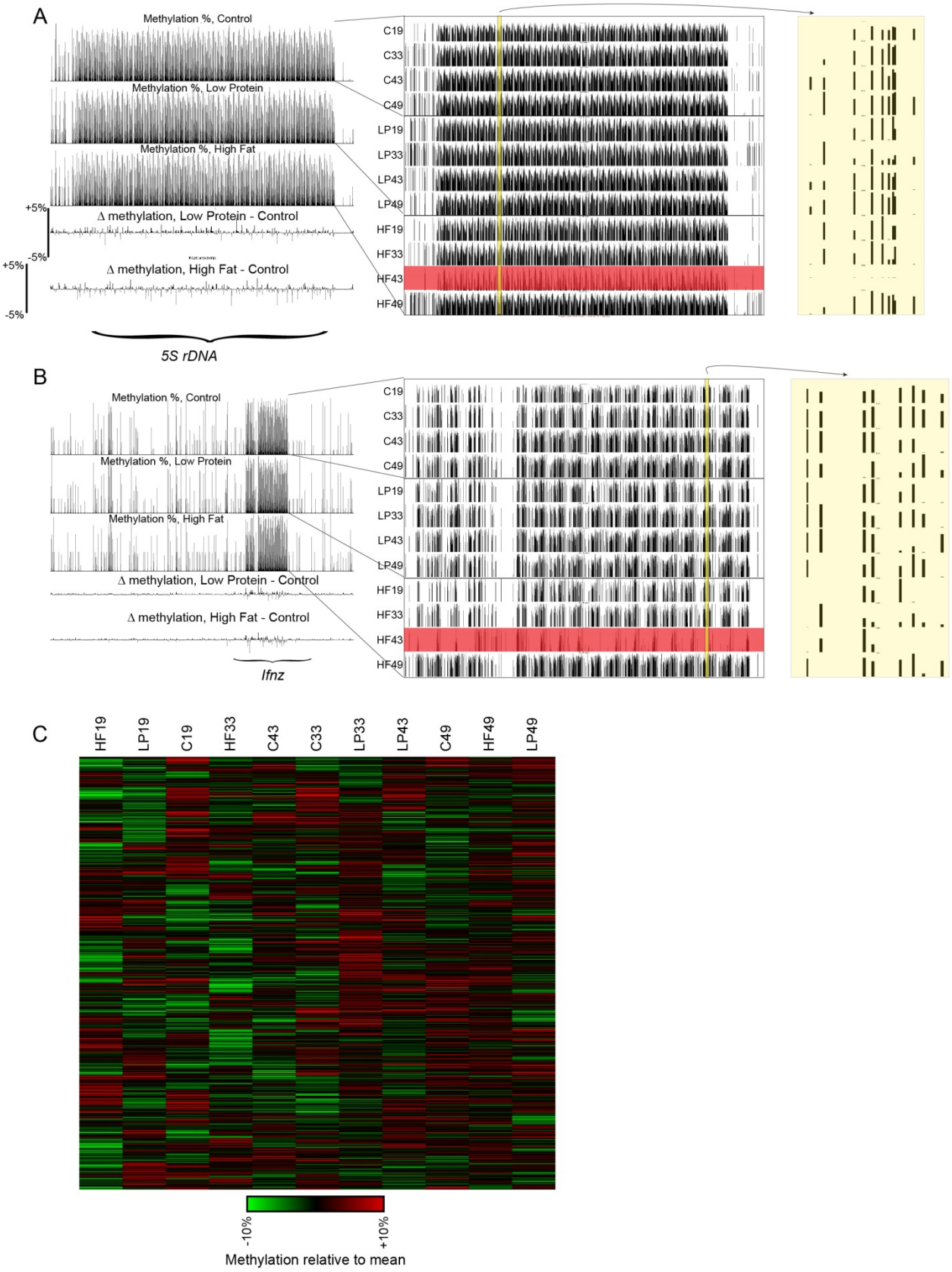
A
Methylation %, Control
Methylation %, Low Protein
Methylation %, High Fat
+5%
Δ methylation, Low Protein - Control
-5%
+5%
Δ methylation, High Fat - Control
-5%
5S rDNA
C19
C33
C43
C49
LP19
LP33
LP43
LP49
HF19
HF33
HF43
HF49
B
Methylation %, Control
Methylation %, Low Protein
Methylation %, High Fat
Δ methylation, Low Protein - Control
Δ methylation, High Fat - Control
Ifnz
C19
C33
C43
C49
LP19
LP33
LP43
LP49
HF19
HF33
HF43
HF49
C
HF19
LP19
C19
HF33
C43
C33
LP33
LP43
C49
HF49
LP49
-10%
+10%
Methylation relative to mean

Figure S3, Related to Figure 2. Epivariation across 11 individual sperm samples. (**A-B**) For each locus (5S rDNA in (A), *Ifnz* cluster in (B)), the left panel shows aggregated data for 4 individual sperm samples for Control, Low Protein, and High Fat animals (methylation is shown from 0 to 100%), with the methylation difference between Low Protein and Control, or High Fat and Control, shown below. Middle panel shows 12 individual sperm samples, grouped by diet. Note that sample HF43 was an extreme outlier in all analyses and so is shown in red. Right panel shows a zoom-in for a particular repeat, as indicated with the yellow box. For both tandem repeat loci, we do not recapitulate the dietary effects observed from pooled WGBS data (see bottom panels in left panel of each), while individual sperm samples confirmed high epivariation at both loci (right panels). (**C**) Epivariation among individual sperm samples. Average methylation was computed for all 300 bp tiles, and the 2000 tiles exhibiting the highest coefficient of variation between 11 sperm samples (excluding HF43) are shown here in a heatmap. For each tile, heatmap shows the difference between the methylation in an individual sample, and the average methylation for that tile across all 11 samples. Both sperm sample and tiles are hierarchically clustered.

Figure S4

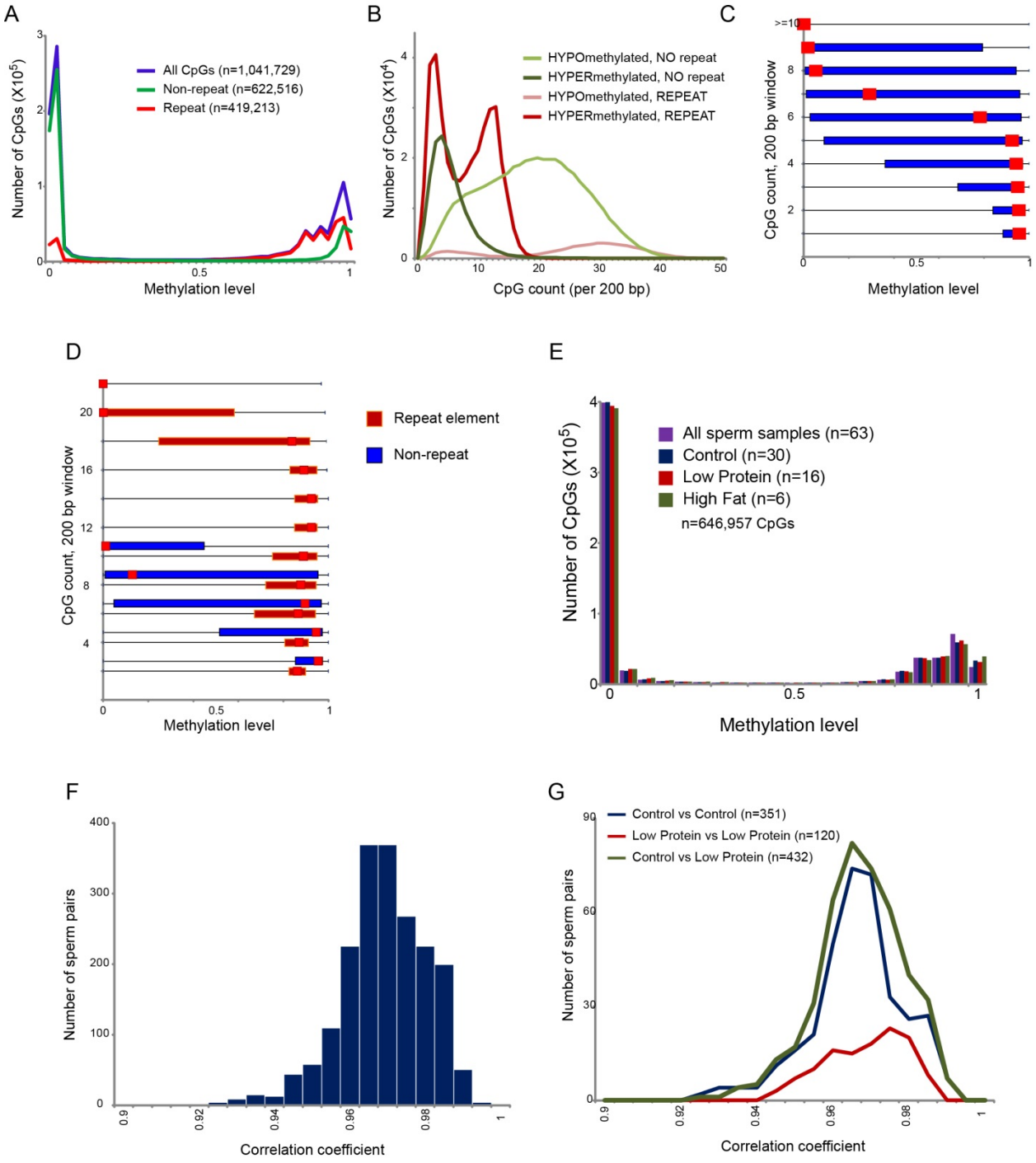

Figure S4, Related to Figure 3. Overview of RRBS dataset. (**A**) Averaged methylation levels for 1,041,729 individual CpGs, which were sequenced at least 10 times in at least one RRBS dataset. Y axis shows number of CpGs exhibiting a given methylation percentage, as indicated on the X axis. Methylation distributions are shown for all CpGs, and separately for CpGs located in annotated repeat elements and for

non-repeat CpGs. (**B**) Relationship between CpG methylation and local CpG density. CpGs were classified as hypomethylated (<20% methylated) or hypermethylated (>20% methylated), and were separated into those located in repeats and those outside repeats. Number of CpGs in each class is shown (Y axis) for CpGs according to their local CpG density (# CpGs in the surrounding 200 bp). (**C**) Boxplots showing the range of methylation values for all CpGs located in various local CpG density regions. Red box shows median, blue bar shows quartiles, and whiskers show max and min. (**D**) As in (**C**), but with CpGs located within and outside of repeat elements plotted separately. (**E**) Methylation distributions are shown as in (**A**) for CpGs averaged across all animals, or across groups of males consuming the indicated diets. Here, only CpGs that were sequenced at least 10 times in at least half of the RRBS datasets (646,957 CpGs) are shown. (**F**) Correlation coefficients were calculated between all possible pairs of RRBS datasets (excluding self-self comparisons which of course would have a correlation of 1), and distribution of correlation coefficients is plotted as a histogram. The number of sperm pairs (y axis) refers to the number of comparisons between pairs of individual sperm samples. (**G**) As in (**F**), but with comparisons shown between pairs of sperm samples isolated from animals on Control diet, between animals on Low Protein diet, or between pairs of animals on Control vs. Low Protein. For each set of sperm samples to be compared, all possible pairs of samples were compared with the exception of self-self comparisons, and the number of comparisons is indicated.

Figure S5

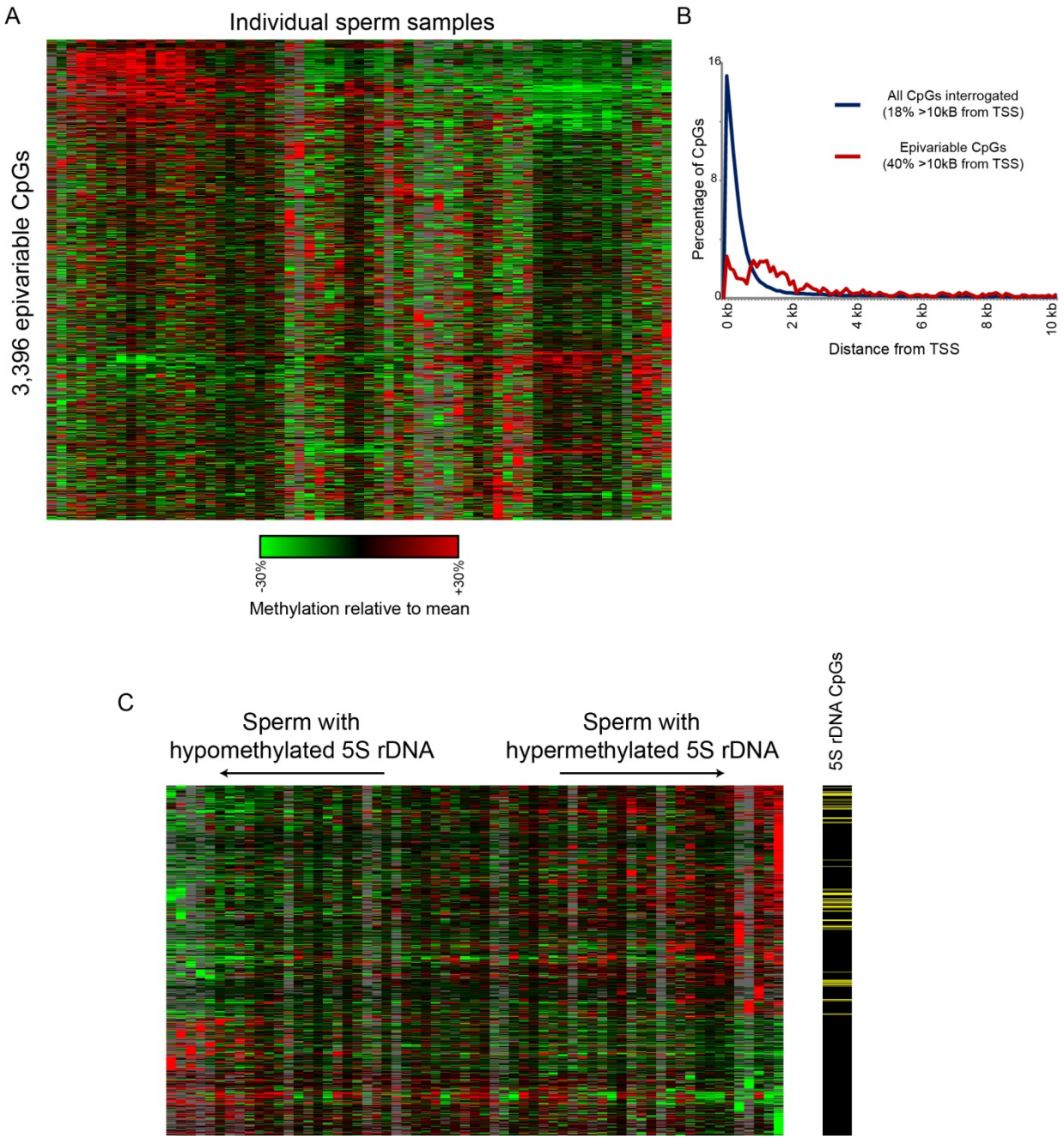

Figure S5, Related to Figure 3. Epivariable CpGs. (**A**) Heatmap of epivariation for 3,396 highly variable individual CpGs. CpGs were selected from non-repeat regions, were sequenced at least 10 times in 80% or more of sperm samples, and deviated from the mean methylation value of that locus by at least 20% in at least 6 individual sperm samples. Data for each CpG are normalized to mean methylation across all animals. (**B**) Distance from epivariable CpGs to the nearest TSS, with distribution of total RRBS dataset shown for background. (**C**) Epivariation at 5S rDNA locus. RRBS data are

shown for all CpGs exhibiting a correlation/anticorrelation of over 0.3/-0.3 to the averaged RRBS data for the 5S rDNA cluster. Multiple CpGs that are unlinked to the rDNA cluster exhibit correlated methylation patterns, consistent with two loci responding either to the same genetic (rDNA copy number) or environmental (number of littermates) cue.

Figure S6

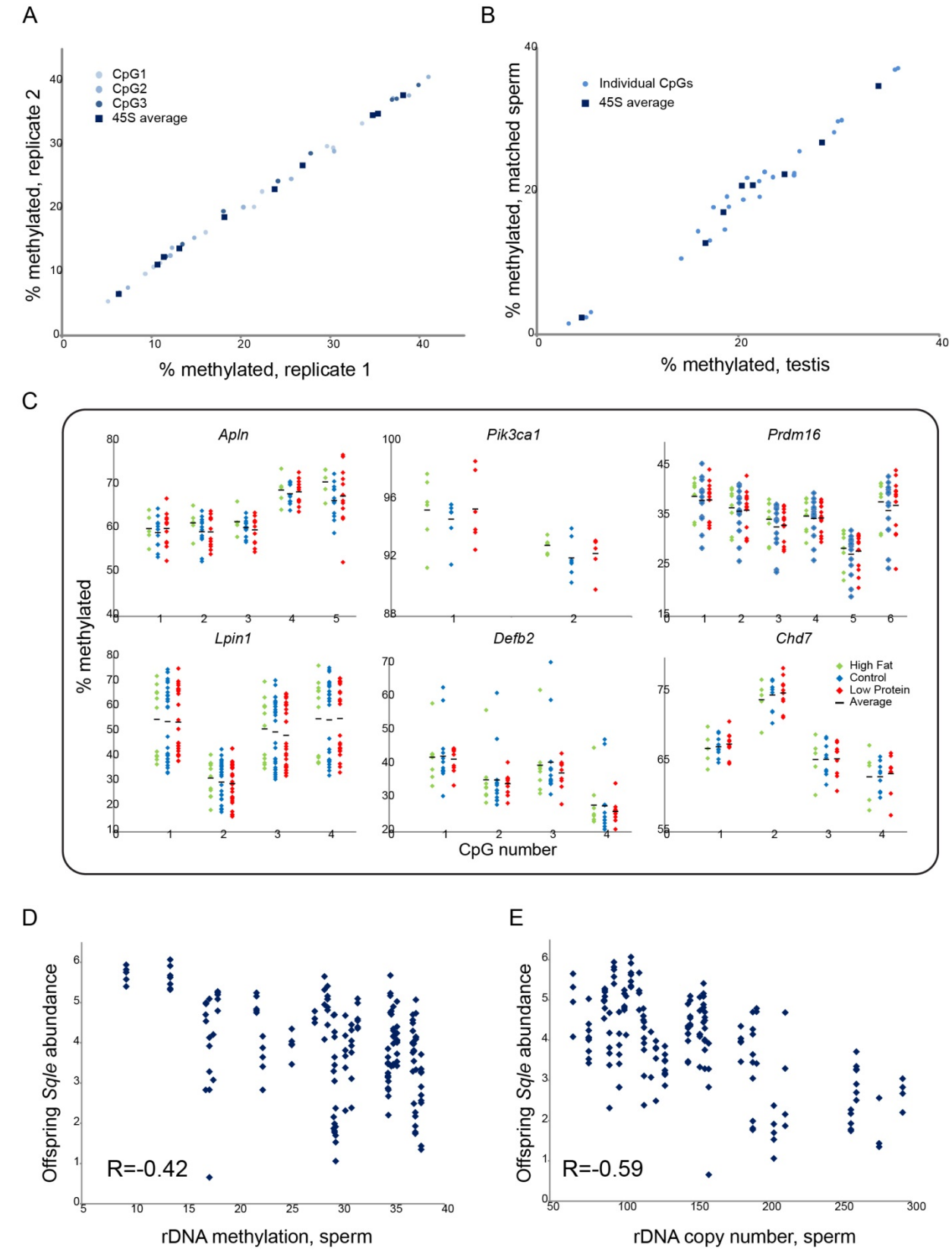
A
CpG1
CpG2
CpG3
45S average
% methylated, replicate 2
% methylated, replicate 1
B
Individual CpGs
45S average
% methylated, matched sperm
% methylated, testis
C
Apln
Pik3ca1
Prdm16
Lpin1
Defb2
Chd7
High Fat
Control
Low Protein
Average
% methylated
CpG number
D
Offspring Sqle abundance
R=-0.42
rDNA methylation, sperm
E
Offspring Sqle abundance
R=-0.59
rDNA copy number, sperm

Figure S6, Related to Figures 4-5. Pyrosequencing analysis of sperm methylation. (**A**) Replicate bisulfite conversions were pyrosequenced, and methylation levels for replicates are scatterplotted for individual CpGs and for the 45S promoter average. (**B**) 45S promoter methylation is highly correlated between sperm and matched testis samples. (**C**) Pyrosequencing of additional loci in murine sperm. Pyrosequencing for six loci which were candidate diet-regulated loci based either on our WGBS dataset, or based on prior reports of dietary regulation in a related paternal effect paradigm (Wei et al., 2014). (**D-E**) Paternal rDNA methylation anticorrelates with offspring cholesterol gene expression. In (**D**), scatterplot shows sperm rDNA methylation level (x axis) vs. expression level of *Sqle* in liver (y axis, shown as 10-(Ct^{Actb}-Ct^{Sqle})) obtained from offspring generated via IVF using an aliquot of the sperm sample used for bisulfite analysis. Y axis in (**E**) shows the same expression data as (D), but with x axis showing rDNA copy number in sperm.

SUPPLEMENTARY TABLES

Table S1, Related to Figures 1-3. Animals used in this study. Dietary and strain information are given for the 61 sperm samples characterized by RRBS, and for the 30 animals used to generate WGBS pools.

Table S2, Related to Figures 1-2. Significantly diet-directed CpG methylation. Significant differentially methylated CpGs were identified with Methylkit (Akalin et al., 2012) using a 300 bp window for assessing the significance of a differentially-methylated CpG. Methylation values for all four WGBS libraries are shown, along with p-values and q-values (FDR-corrected) for Control 1 vs. High Fat and for Control 2 vs. Low Protein. Chromosomal coordinates are from mouse genome build mm9.

Table S3, Related to Figure 2. Methylation levels over repeat elements. Averaged methylation levels over Repeatmasker elements for the 4 WGBS libraries, as indicated.

PRIMERS USED IN THIS STUDY. Primers for bisulfite pyrosequencing, for q-PCR, and for q-RT-PCR are shown, along with original reference if any (Carone et al., 2010; Shiao et al., 2012; Wei et al., 2014).

Primer Name	Target	Assay	Sequence	Reference
Pyrosequencing Primers				

45S_rDNA_Bio-BF2	45S rDNA	Pyro	Bio-GAATTTGATTATTTAGAGGAAGTAAAAGT	
45S_rDNA_BR2	45S rDNA	Pyro	CACCCACCCCTTCTCT	
45S-rDNA_Seq2	45S rDNA	Pyro	CTCACTCCAAACACC	
45s_Spacer_BF2	45S rDNA	Pyro	GGAAGTGTTTGTGGTGAGG	Shiao *et al.*
45s_Spacer_Bio_BR2	45S rDNA	Pyro	BIO-CACCAACCCTAACATTTTTCC	Shiao *et al.*
45s_Spacer_Seq3	45s rDNA	Pyro	GTTTTGGAGATGGTGT	Shiao *et al.*
Rex2_Bio-BF1	Rex2	Pyro	Bio-GGATTTTTTTTTGTTTTTTAGGTGATT	
Rex2_BR1	Rex2	Pyro	AACCCAAACTACTTCCCT	
Rex2_Seq1	Rex2	Pyro	CCAAACTACTTCCCTC	
Pik3ca1_BF1	Pik3ca1	Pyro	AGTGAGGAGAAATAAGATTAAAGTAAGA	Wei *et al.*
Pik3ca1_Bio-BR1	Pik3ca1	Pyro	Bio-ACCTAATTCTACAACTTACATCCATAAAT	
Pik3ca1_Seq1	Pik3ca1	Pyro	AGTAAGATTGAAATTTAATAGAGT	
Prdm16-BF1	Prdm16	Pyro	GTGATTTTTTTTAAAAGGGTATATAAGGAG	
Prdm16-Bio-BR1	Prdm16	Pyro	Bio-CCCTCCCCCAAATCTACTAAA	
Prdm16-Seq1	Prdm16	Pyro	AAAGGGTATATAAGGAGAG	
Apln-BF1	Apln	Pyro	AGTATTTGGTATAGTAGTTAATATAGGAGA	
Apln-Bio-BR1	Apln	Pyro	Bio-CTATCACCAAAATCAATACCAATTTA	
Apln-Seq1	Apln	Pyro	ATTGGTTTTTATTATTATGGTTAAG	
Chd7-BF1	Chd7	Pyro	AAGGATTATAGGTTTTGAGGATAAGTA	
Chd7-Bio-BR1	Chd7	Pyro	Bio-AAAACCTTAATCTATAAACATCTACTAACC	
Chd7-Seq1	Chd7	Pyro	GGATTTAGGGATGATGAG	
Lpin1-BF1	Lpin1	Pyro	GTGGTAGAGATTTATGAGGATTATAAG	
Lpin1-Bio-BR1	Lpin1	Pyro	Bio-AAACTTTAAAATACCAAAAACTTTTC	

			C	
Lpin1-Seq1	Lpin1	Pyro	ATATAGTGGAGGTTATTTAGTTTAG	
DefB-BF1	DefB	Pyro	AGATTTTGGGAGTTGTGGTAGT	
DefB-Bio-BR1	DefB	Pyro	Bio-CCCCACCAACACAACATCCTTA	
DefB-Seq1	DefB	Pyro	GGAGTTGTAGTTTGGGGA	
ddPCR				
45S rDNA F1	45S rDNA	ddPCR	TTCTCTTGTTCTGTGTCTGCC	
45S rDNA R1	45S rDNA	ddPCR	GGGAGAAACAAGCGAGATAGG	
45S rDNA Probe	45S rDNA	ddPCR	AGTAACTGTCTTGCCCCGCGT	
Gapdh F1	Gapdh	ddPCR	AGCCTTTACTACAGAACATCTCAC	
Gapdh R1	Gapdh	ddPCR	TCTTTCCTCTCCCTTCCCTTTA	
Gapdh Probe	Gapdh	ddPCR	ACTCCAACA/ZEN/AATGCTTGCTGACGC	
CNV qPCR				
rDNA_Term_F1	45S rDNA	qPCR	GAACCTTTAGGTCGACCAGTTG	
rDNA_Term_R1	45S rDNA	qPCR	ACAAAGTACCACCCGGAGTA	
Lys(CTT)_F1	Lys tDNA	qPCR	CGGCTAGCTCAGTCGGTAGA	
Lys(CTT)_R1	Lys tDNA	qPCR	AACCCACGACCCTGAGATTA	
Acacb_F1	Acacb	qPCR	TGCTCATAGGCCAAGAGAAAGGCT	
Acacb_R1	Acacb	qPCR	AGTGCTGGGATTACAGGCATGAGT	
5S rDNA F2	5S rDNA	qPCR	CCCGATCTCGTCTGATCTC	
5S rDNA R2	5S rDNA	qPCR	CCTACAGCACCCGGTATT	
qPCR Primers				
Sqle_F1	Sqle	RT-qPCR	TCCTTGCATCAGCTCCGAAA	Carone *et al.*
Sqle_R1	Sqle	RT-qPCR	CGGTCAAAGCAACCCAACAGGACCG	Carone *et al.*
B-Actin_F1	B-Actin	RT-qPCR	GGCTGTATTCCCCTCCATCG	
B-Actin_R1	B-Actin	RT-qPCR	CCAGTTGGTAACAATGCCATGT	

SUPPLEMENTARY REFERENCES

Akalin, A., Kormaksson, M., Li, S., Garrett-Bakelman, F.E., Figueroa, M.E., Melnick, A., and Mason, C.E. (2012). methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. Genome biology *13*, R87.

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Wei, Y., Yang, C.R., Wei, Y.P., Zhao, Z.A., Hou, Y., Schatten, H., and Sun, Q.Y. (2014). Paternally induced transgenerational inheritance of susceptibility to diabetes in mammals. Proceedings of the National Academy of Sciences of the United States of America *111*, 1873-1878.