Supplementary Information

Supplementary Tables

Table S1 – Chromsomal coordinates of differentially methylated regions, determined by MEDIPS, between E3.5 uteri and E11.5 decidua

Click here to Download Table S1

Table S2 – List of primers used

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Table S3 – Source Data for Figs. S3A, S3B: RT-qPCR values for *Fzd2*, *Hoxa10*, *Hoxa11* and, *Tet1*

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Table S4 – Source Data for Fig. 3E: RPM values for *Tet1*

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Fig. S1

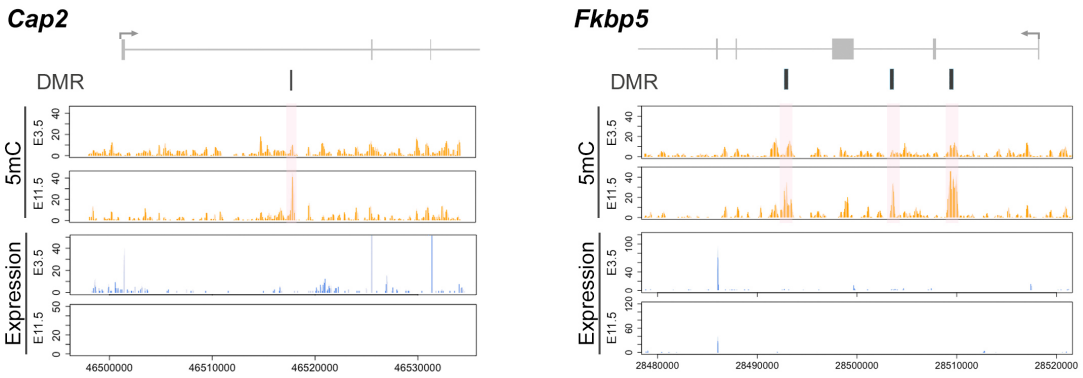

Fig. S1

Additional examples of the *Cap2* and *Fkbp5* gene loci where a gain of DNA methylation (5mC) at identified DMRs (shaded) correlates with lower gene expression at E11.5 compared to E3.5. Wiggle plots of read count enrichments across these regions are shown.

Fig. S2

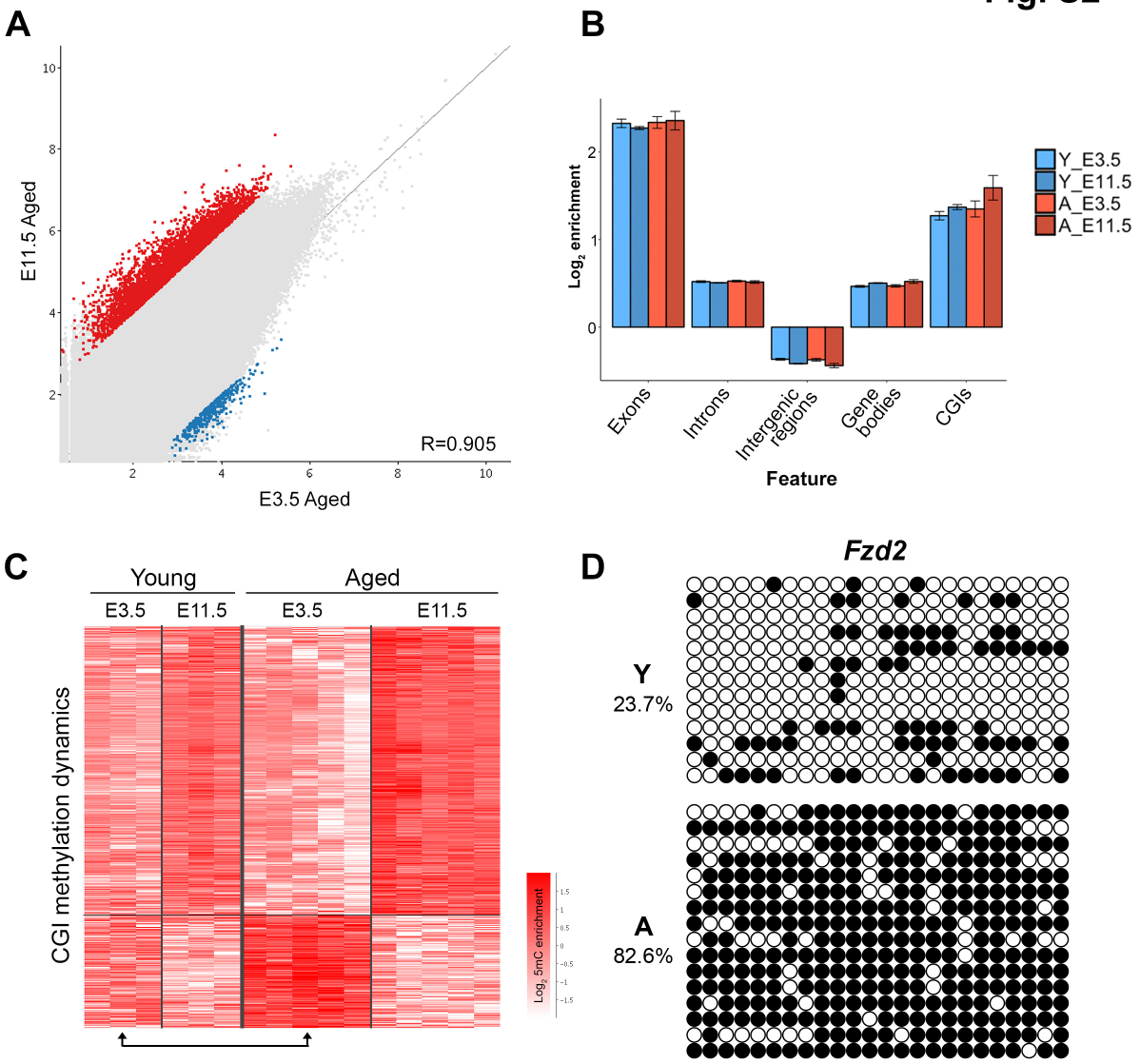

Fig. S2

(A) Scatter plot of meDIP-seq data of E3.5 uteri and E11.5 decidual samples from aged females (n=5 each). Each dot represents a 2kb tiling probe, overlapping by 1kb, across the entire genome. Differentially enriched probes (Log_2 +/- 2) are colour-coded, with blue and red dots representing genomic regions that are hypo- and hypermethylated at E11.5, respectively. (B) DNA methylation enrichment across various genomic features in E3.5 uteri and E11.5 deciduas of young ("Y") and aged ("A") females. A higher enrichment of DNA methylation is evident at CGIs of aged E11.5 samples. (C) Heatmap of DNA methylation enrichment, determined by meDIP-seq, at CpG islands (CGIs) that are differentially methylated between aged E3.5 and E11.5 samples. These dynamically regulated CGIs show the same trends of gain and loss of methylation in the young samples, but to a less pronounced extent. Note in particular that CGIs that lose methylation during decidualisation are markedly hypermethylated in E3.5 aged uteri (highlighted by arrow). (D) Verification of differential DNA methylation at the *Fzd2* CGI performed by standard bisulphite sequencing. Each circle represents a CpG dinucleotide, with filled circles representing methylated and open circles unmethylated cytosines, respectively. Each line depicts an independent allele, all sequences were checked for the absence of clonal amplification. Input DNA was from 3 young ("Y") and 4 aged ("A") E3.5 uteri.

Fig. S3

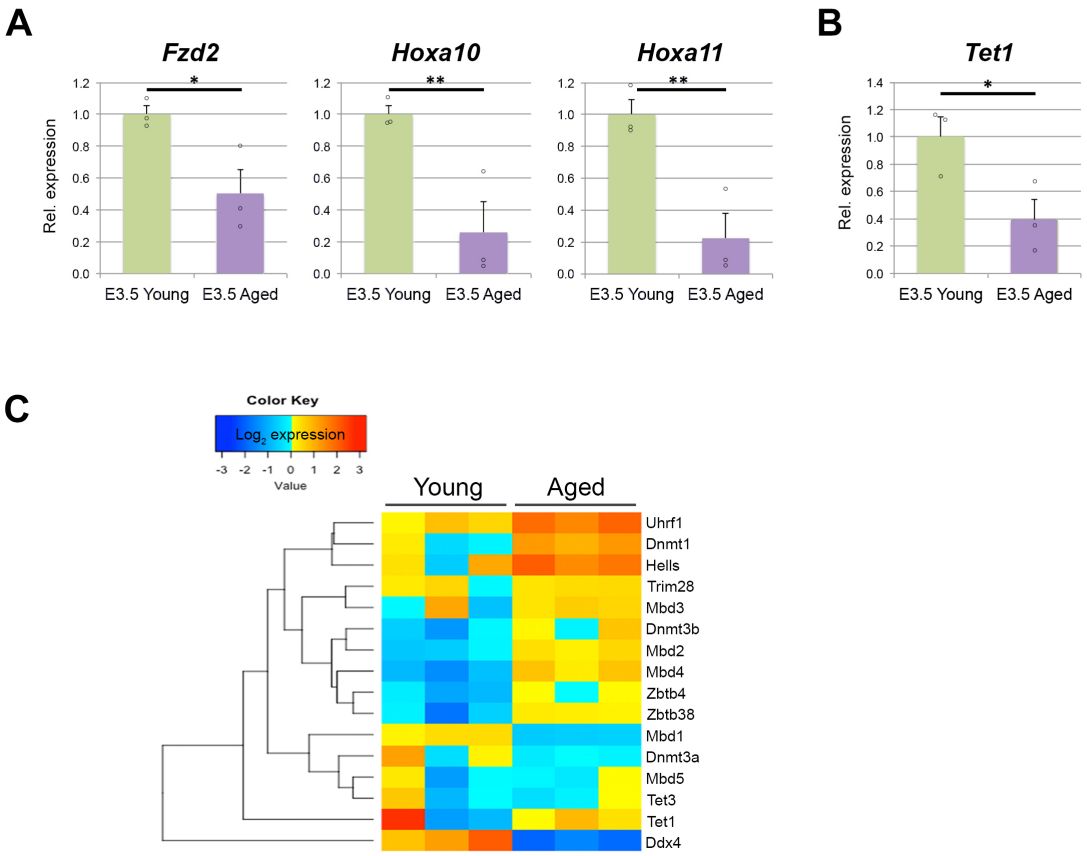

Fig. S3

(A) and (B) RT-qPCR validation of RNA-seq data confirming the differential expression of *Fzd2*, *Hoxa10*, *Hoxa11* (A) and *Tet1* (B) in aged versus young E3.5 uteri (n=3 each, mean +/- s.e.m.). **p*<0.05; ***p*<0.01 (t-test). (C) Heatmap of RNA-seq data generated from primary stromal cells isolated from E3.5 uteri of young and aged females. Displayed are differentially expressed DNA methylation machinery components; notably, *Dnmt1*, *Dnmt3b*, *Uhrf1* and *Trim28* (=*Kap1*) are expressed at significantly higher levels in uterine stromal cells from aged compared to young females.