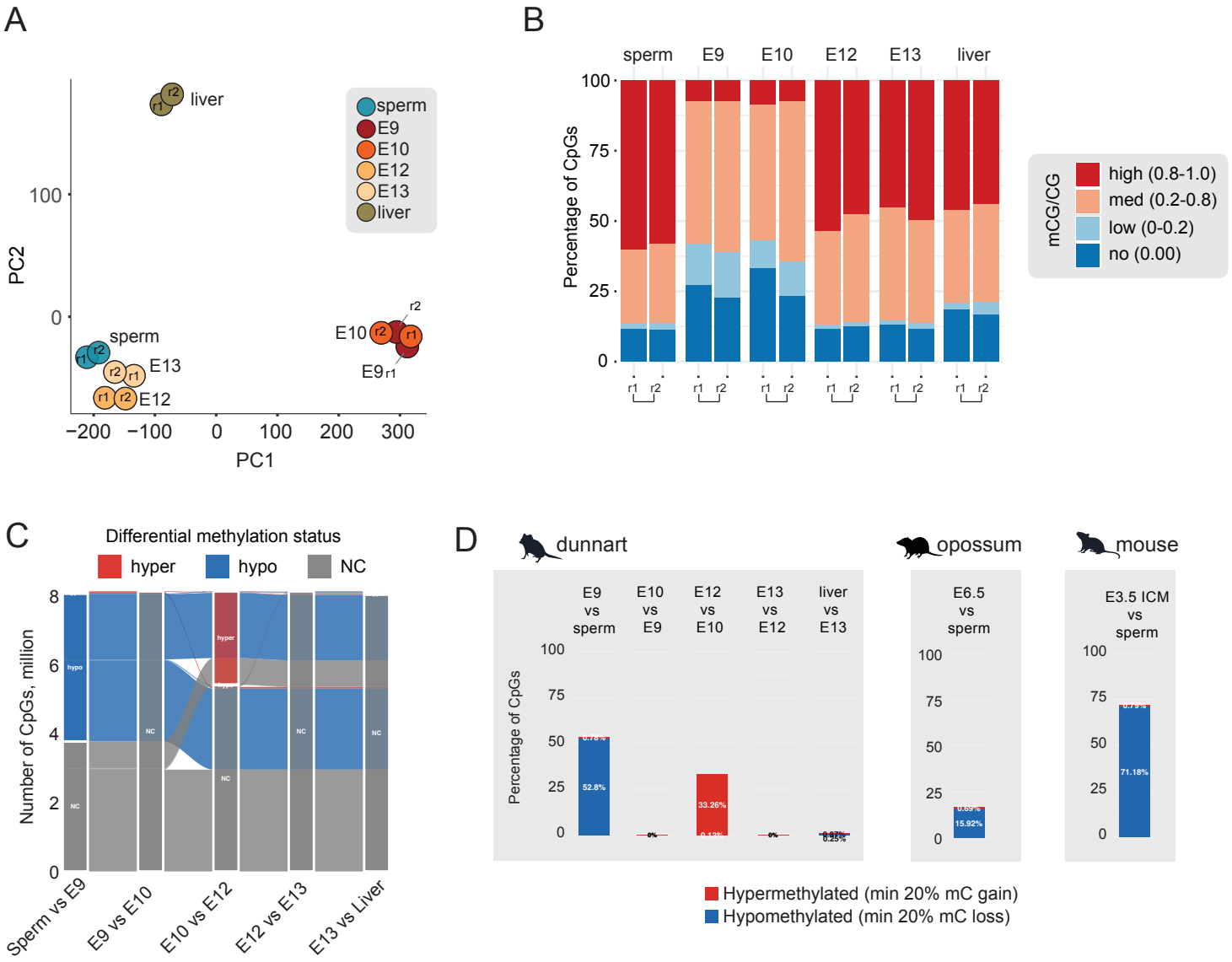
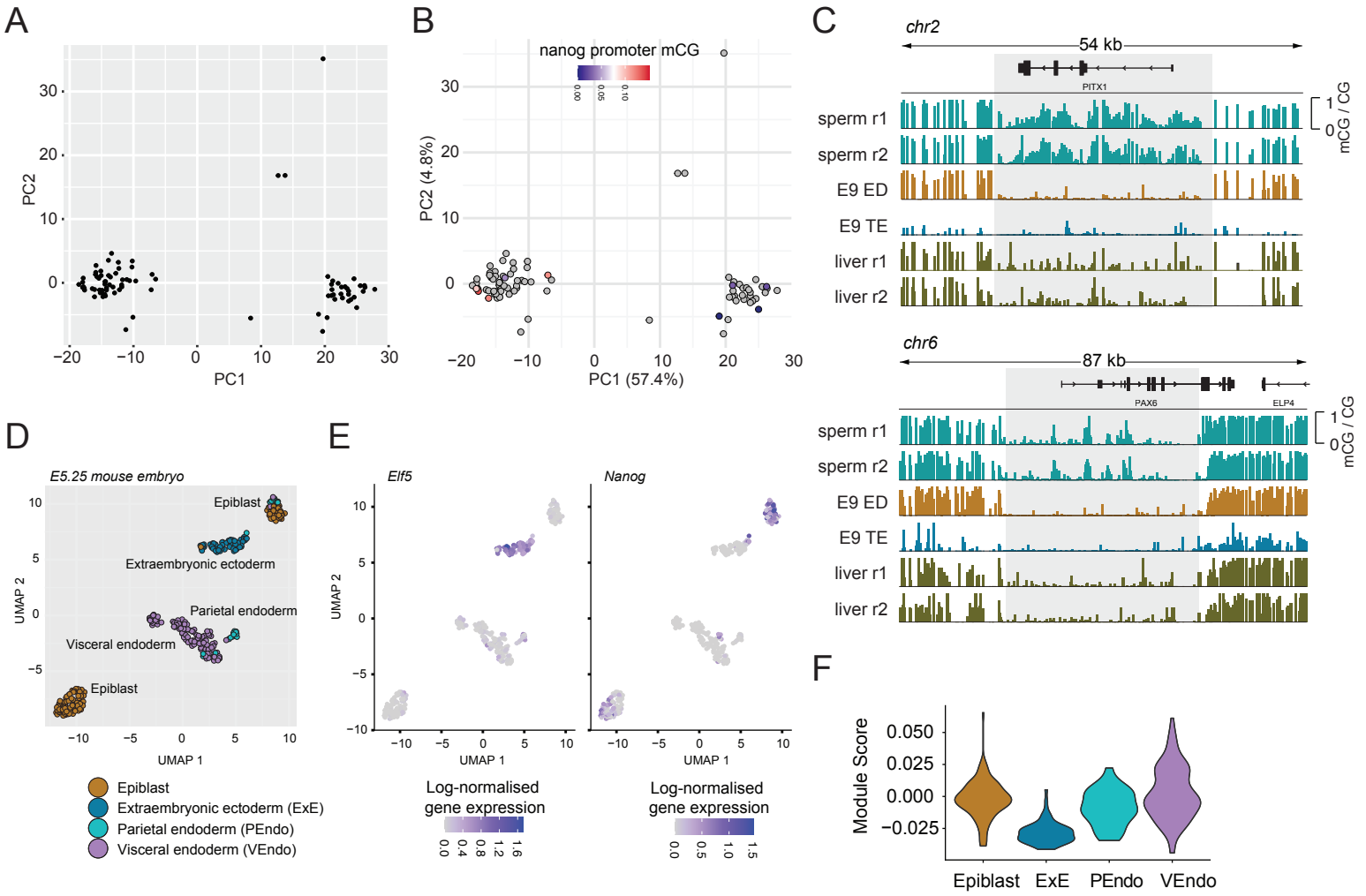
Supplementary Figure 1 Angeloni et al



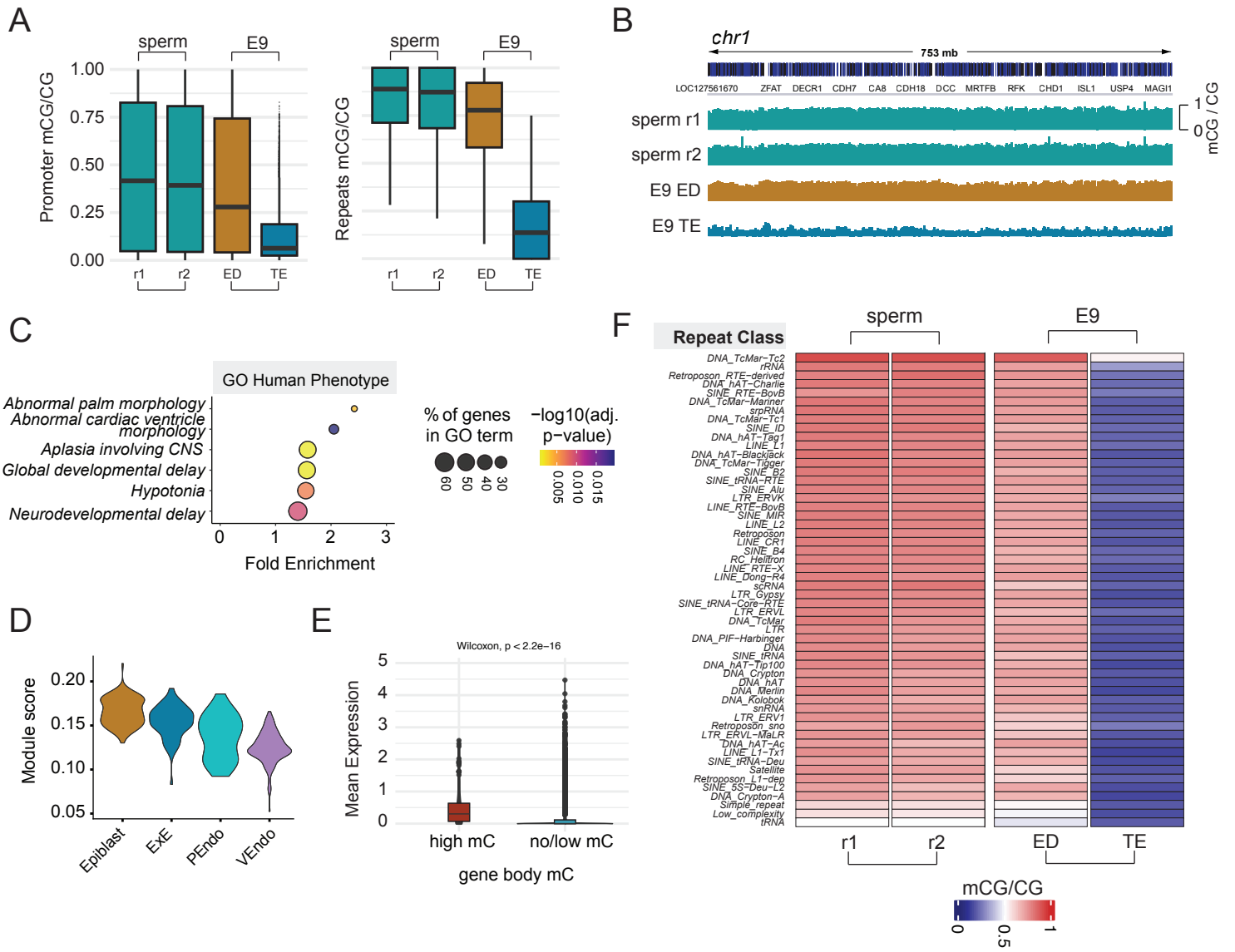
Supplementary Figure 1: DNA methylation dynamics in the fat-tailed dunnart embryo. (A) Principal component analysis (PCA) of dunnart methylomes binned into 1kb regions. **(B)** Stacked bar plots indicating the percentages of methylated CpG sites in dunnart sperm, embryos and adult tissue. High, 80-100%; medium, 20-80%; low, >0-20%; no, 0% methylation. **(C)** Alluvial plot showing the number and dynamics of hypo- and hypermethylated differentially methylated cytosines (DMCs) between consecutive developmental stages. **(D)** Percentages of differentially methylated CpGs out of all covered CpGs in the genome between consecutive stages of embryonic development in the dunnart, opossum and mouse. CpGs with at least 20% methylation loss compared to the previous stage were considered as hypomethylated. CpGs with at least 20% methylation gain compared to the previous stage were considered as hypermethylated.

Supplementary Figure 2 Angeloni et al



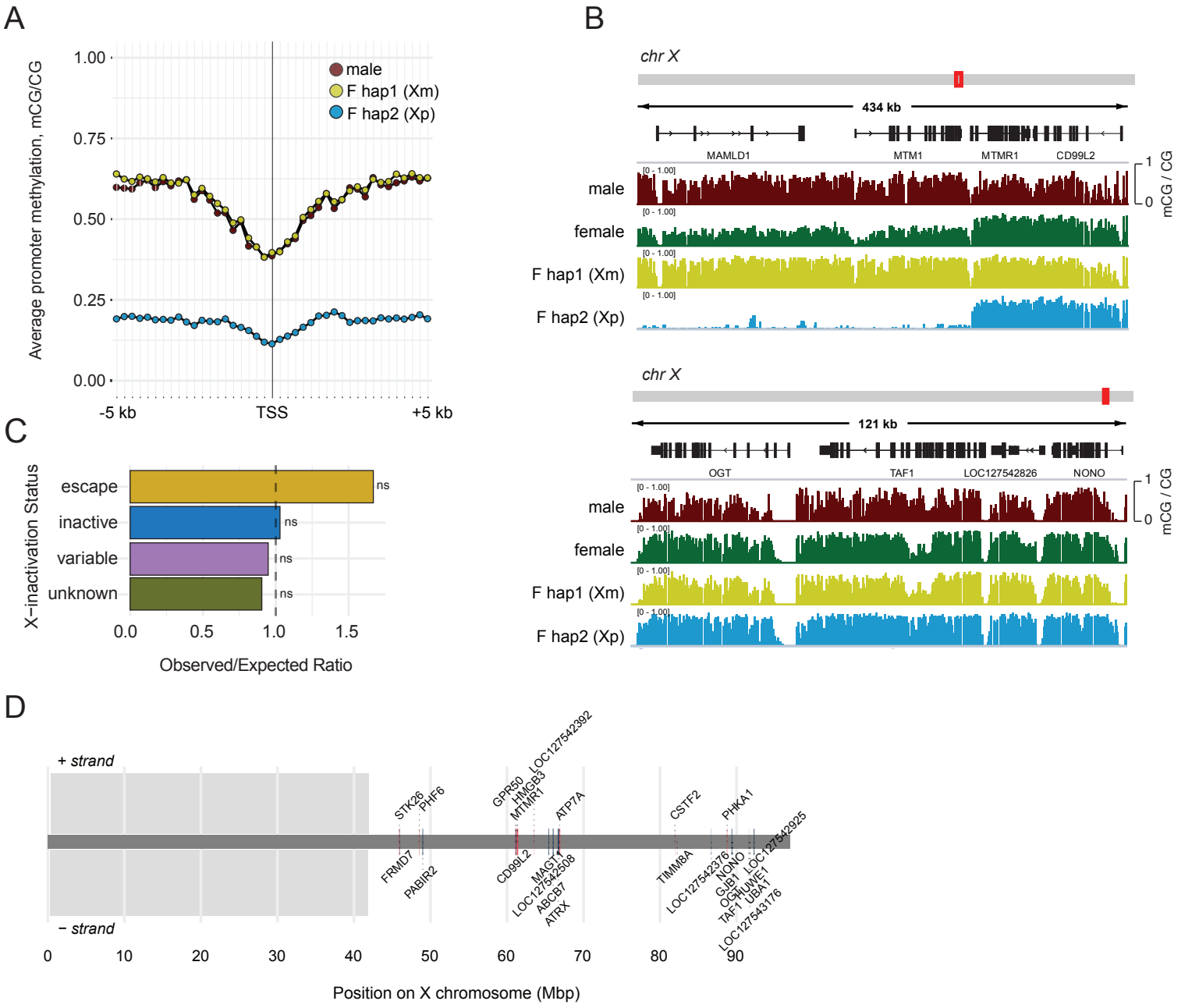
Supplementary Figure 2: Single cell DNA methylation analysis of embryonic and extraembryonic lineages in the E9 dunnart embryo. (A) Principal component analysis (PCA) of single cell promoter DNA methylation in the E9 dunnart embryo. **(B)** PCA of single cell promoter DNA methylation in the E9 dunnart embryo, depicting per-cell mean methylation of the embryonic marker gene promoter Nanog. Cells coloured in grey lack sufficient read coverage for promoter of interest. **(C)** IGV browser track depicting sperm and liver EM-seq as well as pseudobulked E9 embryo's TE and ED scBS-seq DNA methylation profiles. **(D)** Uniform manifold approximation and projection (UMAP) of 331 individual cells from the E5.25 mouse embryo. Cells are color-coded by tissue types. **(E)** UMAP showing expression levels of the extraembryonic ectoderm marker Elf5 and the epiblast marker Nanog. **(F)** Violin plots showing module scores of the gene set that undergoes DNA methylation reprogramming in embryonic and extraembryonic lineages. Module scores depict the difference between the average gene expression of the gene set and random control genes. ExE - extraembryonic ectoderm, PEndo - parietal endoderm, VEndo - visceral endoderm.

Supplementary Figure 3 Angeloni et al



Supplementary Figure 3: Genome-wide DNA methylation erasure in the dunnart's trophectoderm. (A) Boxplots showing average promoter and repetitive elements DNA methylation levels in sperm and E9 embryo's trophectoderm (TE) and embryonic disk (ED). **(B)** IGV browser track depicting sperm EM-seq and pseudobulked E9 embryo's TE and ED scBS-seq DNA methylation profiles. The entire chr1 is shown. **(C)** Gene ontology (GO) analysis of the genes that retain high mC levels in TE. The human phenotype ontology results are shown. **(D)** Violin plots showing module scores of the gene set with high DNA methylation in TE. Module scores depict the difference between the average gene expression of the gene set and random control genes. ExE - extraembryonic ectoderm, PEndo - parietal endoderm, VEndo - visceral endoderm. **(E)** Boxplots showing mean expression levels of genes that retain high gene body DNA methylation vs genes that undergo DNA methylation erasure at their gene bodies. **(F)** Heatmap showing DNA methylation at transposable elements in sperm, E9 embryo's trophectoderm (TE) and embryonic lineage (ED).

Supplementary Figure 4 Angeloni et al



Supplementary Figure 4: DNA methylation profiling of active and inactive X chromosomes and escape from X chromosome inactivation in the fat-tailed dunnart. (A) Promoter DNA methylation profiling of the active maternal (yellow) and inactive paternal X chromosomes in the female, as well as single male X chromosome (red) in the adult liver. **(B)** IGV browser track showing examples of genes that exhibit unusually high DNA methylation levels on the inactive paternal X chromosome in female dunnart liver. **(C)** Observed over expected enrichment of highly methylated dunnart genes on the inactive Xp among known human escapee, variable and inactive X-linked genes. **(D)** Location of putative fat-tailed dunnart escapee genes on the X chromosome.