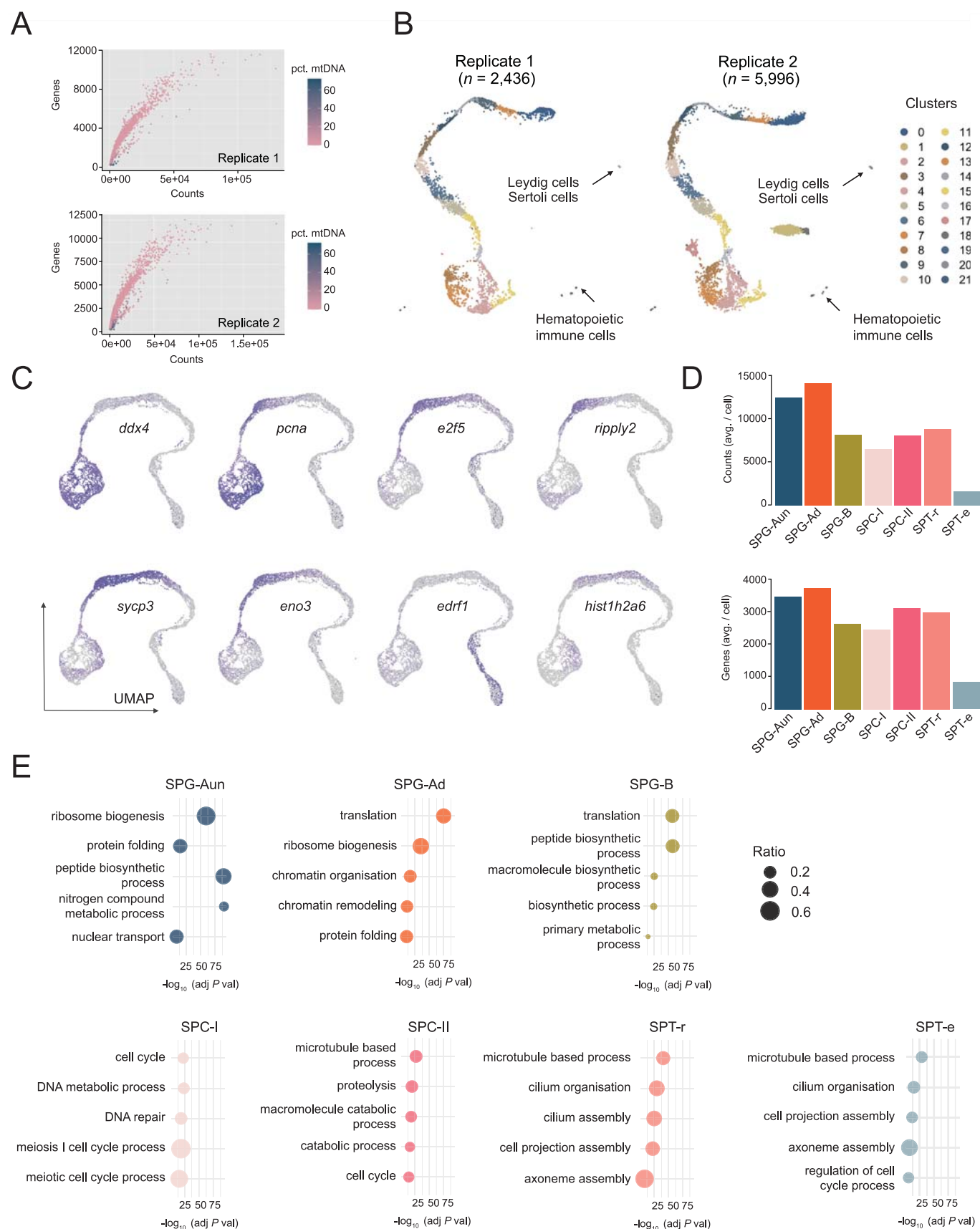


Expanded View Figures

Figure EV1. scRNA-seq quality control, marker genes and GO enrichments.

(A) Quality control metrics for scRNA-seq replicates showing the relationship between counts, detected gene numbers, and mitochondrial DNA percentage. (B) UMAP (uniform manifold approximation and projection) plots of the zebrafish testis tissue denoted by Seurat clusters before filtering. (C) Distribution of average counts, and average gene numbers across the spermatogenic lineage. (D) Expression UMAP for canonical marker genes for SPG (*ddx4*), SPG-Aun (*eno3*, *e2f5* and *rippy2*), SPG-Ad(*hist1h2a6*), SPT-e (*edrf1*), SPC (*sycp3*) and proliferation (*pcna*). (E) Dot plot of the top 5 GO biological processes enriched using marker genes for each cell type. The x-axis shows adjusted *P* values (FDR), and dot size reflects the ratio of intersected to total terms. Enrichment *P* values were computed with Fisher's exact one-tailed test (cumulative hypergeometric).



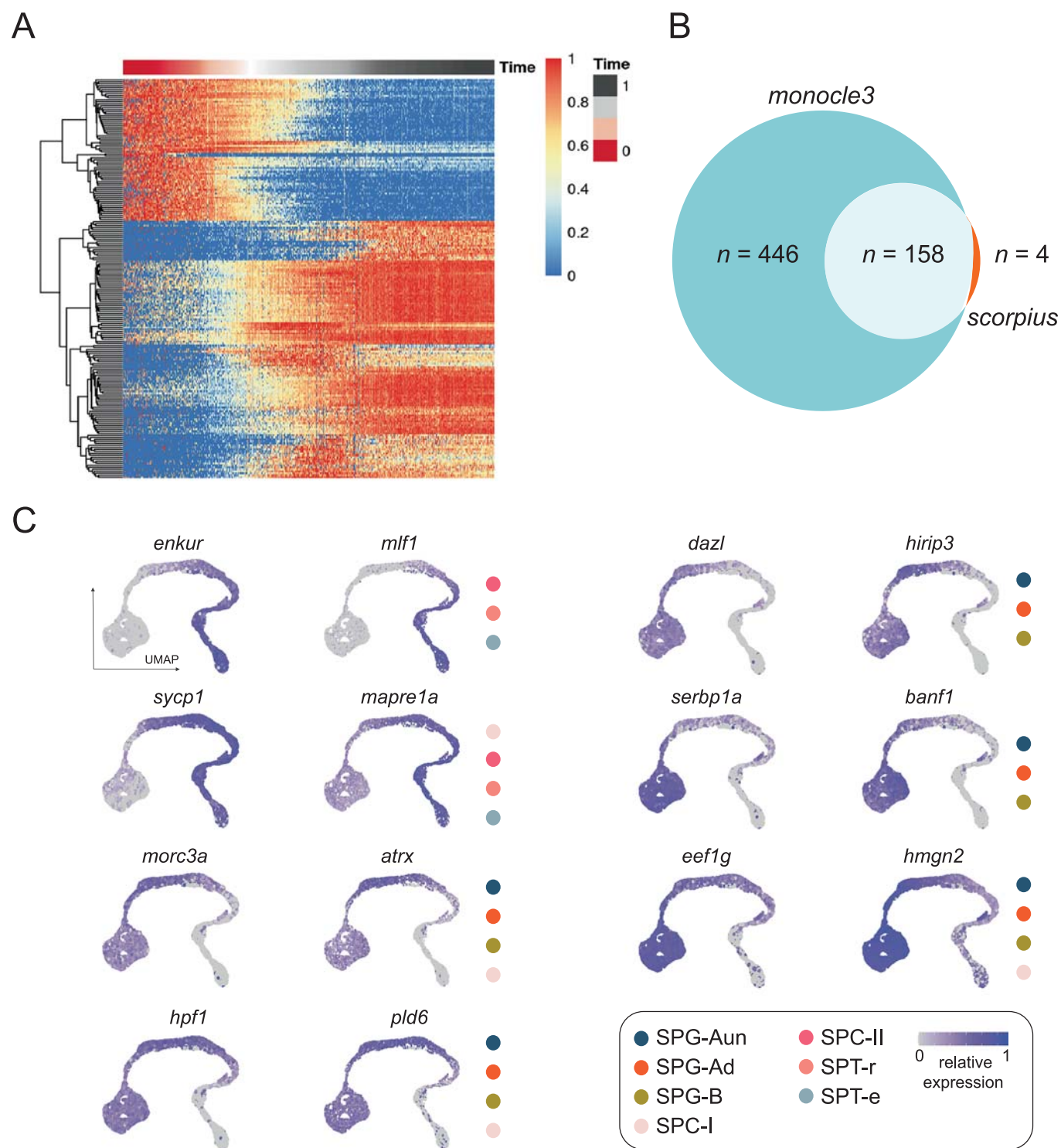


Figure EV2. Trajectory analysis and expression profiles of spermatogenesis driver genes.

(A) Heatmap of drive genes across the inferred trajectory of spermatogenesis. Samples ranked by pseudotime trajectory inferred by SCORPIUS. Rows represent genes grouped by hierarchical clustering with distinct expression patterns. (B) Comparison of gene drivers for spermatogenesis calculated using Monocle3 and SCORPIUS software. (C) UMAP examples for gene drivers obtained from the trajectory analysis identified by both Monocle3 and SCORPIUS.

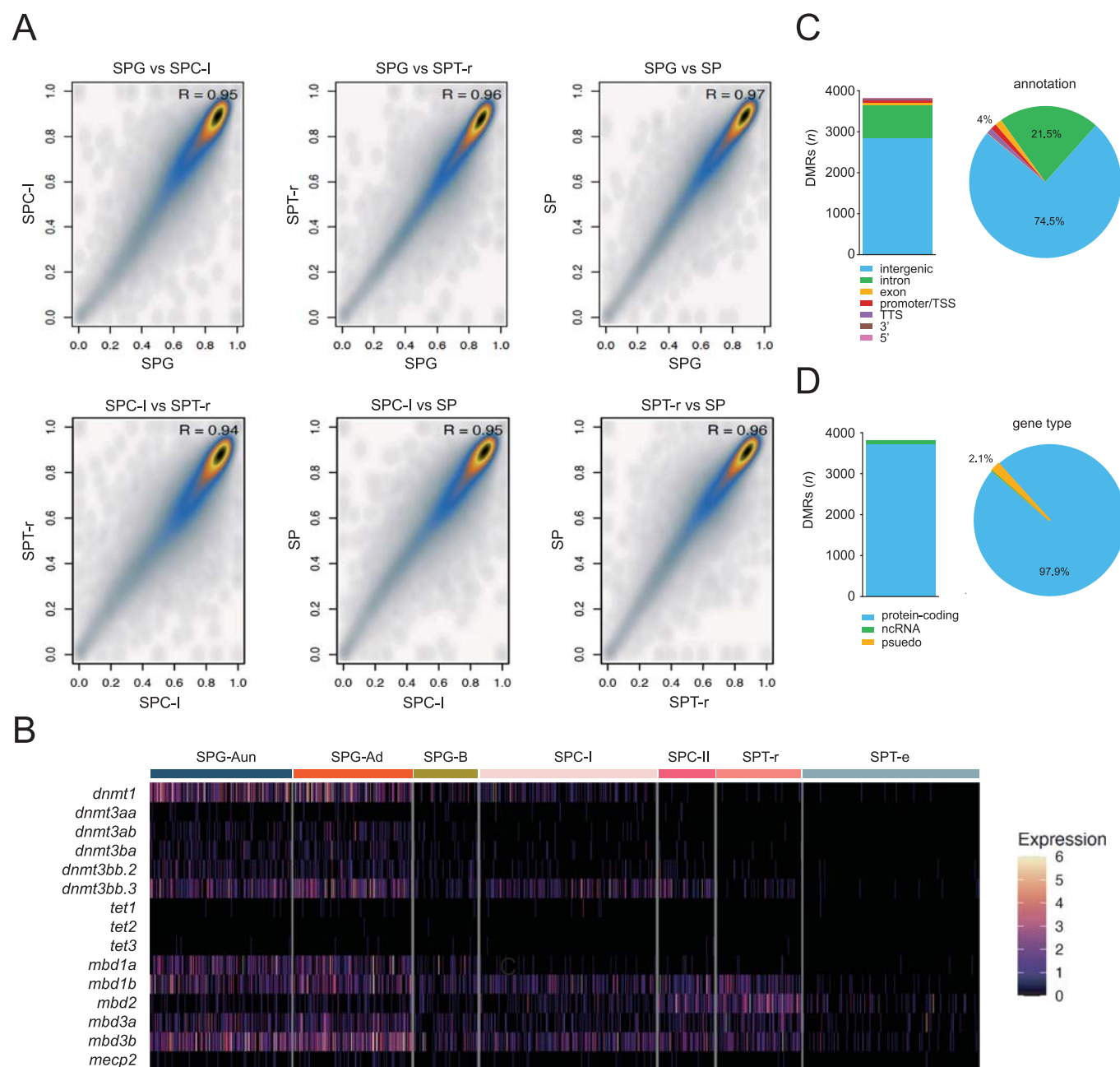
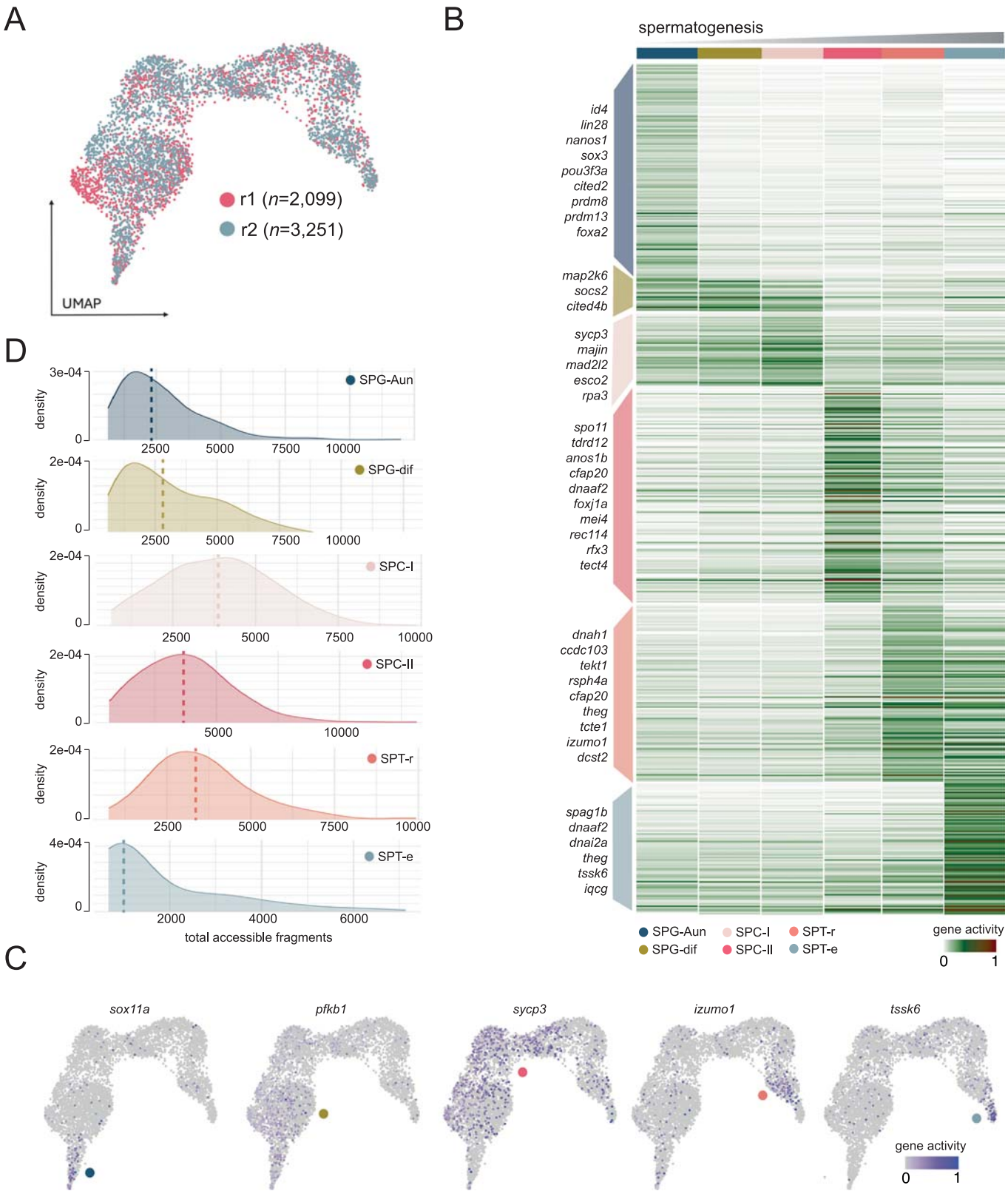


Figure EV3. DNA methylomes and genomic localisation of Differentially Methylated Regions (DMRs).

(A) Correlation of CpG methylation levels in non-overlapping 10 Kb genomic bins in isolated germ cell populations (SPG: spermatogonia, SPC-I: spermatocytes I, SPT-r: round spermatids, and mature sperm: SP). R value represents Pearson Correlation coefficient. SPG vs SPC-I, SPC-I vs SPT-r, and SPT-r vs SP comparisons are also shown in Fig. 3E. (B) Single-cell RNA-seq expression of DNA methylation, deposition removal, and interpretation machinery during zebrafish spermatogenesis. Genes include DNA methyltransferases, TET dioxygenases, and methyl-CpG-binding domain proteins. Expression is shown across major germ cell populations, ordered by developmental progression from undifferentiated spermatogonia to mature spermatids. (C) Genomic localisation of DMRs ($n = 3879$) represented as absolute values (left panel) and percentages (pie chart, right panel) identified between four germ cell populations. (D) Gene type enrichment of the identified DMRs.



◀ **Figure EV4. Single cell chromatin accessibility (scATAC-seq) dynamics during zebrafish spermatogenesis.**

(A) UMAP (uniform manifold approximation and projection) of biological replicates of scATAC-seq data. Cells are coloured by replicate label. (B) Sorted heatmap of averaged gene expression profiles grouped by cell type (SPG-Aun, SPG-dif, SPC-I, SPC-II, SPT-r, SPT-e). Top marker genes for each cluster are indicated on the left-hand side of the heatmap. (C) UMAP plots showing gene activity of marker gene examples associated with spermatogenesis: *sox11* - undifferentiated spermatogonia; *pfkb1* - differentiated spermatogonia; *sycp3* - spermatocytes; *izumo1* - round spermatids; *tssk6* - elongated spermatids. Gene activity is defined as the normalised sum of fragments spanning the gene body and extending 2 kb upstream of the transcription start site. (D) Distribution of the number of accessible genome fragments in each cell type (SPG-Aun, SPG-dif, SPC-I, SPC-II, SPT-r, SPT-e). The median is indicated by the dashed line.



Figure EV5. Chromatin state of elongated spermatids (SPT-e).

(A) Dot plot of top biological processes associated with genes linked to open chromatin peaks in the SPT-e population. The x-axis shows adjusted *P* values (FDR), and dot size reflects the ratio of intersected to total terms. Enrichment *P* values were computed with Fisher's exact one-tailed test (cumulative hypergeometric). (B) Top 12 enriched motifs found in open chromatin peaks in each cell population. (C) ATAC-seq signal (log2) from all spermatogenesis cell types, plotted over unmethylated regions (UMRs) identified in mature sperm.