

Table S1. Prepubertal testis single-cell RNA-seq datasets.

	Guo et al., 2018 [44]	Sohni et al., 2019 [42]	Guo et al., 2020 [43]	Hermann et al., 2018 [33]	This Study	This Study
Age	(infant) 12 and 13 months	(newborn) 2 and 7 days	(juvenile) 7 and 11 years	Postnatal day (P) 6	15 and 20 months	Postnatal day 0, 26 months
# Testis cells	2245 Infant1_1- 568 Infant1_2- 467 Infant2_1- 597 Infant2_2- 613	24,780 D2_I— 2353 D2_T— 6490 D7_I— 11,767 D7_T— 4170	3727 11 yo_rep1— 1069 11 yo_rep2— 700 7 yo_rep1— 991 7 yo_rep2— 967	13,554 Bright—5552 Dim—4391 Unselected— 3611	23,219 B36506A— 9306 B36506B— 5921 B37979—7992	25,342 R34403— 9935 R37136A— 8253 R37136B— 7154
Mean Reads per Cell	Infant1_1- 79,287 Infant1_2- 91,489 Infant2_1- 131,936 Infant2_2- 137,661	D2_I— 31,753 D2_T— 21,702 D7_I— 21,792 D7_T— 25,900	11 yo_rep1— 166,257 11 yo_rep2— 44,427 7 yo_rep1— 86,716 7 yo_rep2— 129,258	Bright— 108,179 Dim— 117,390 Unselected— 160,375	B36506A— 62,329 B36506B— 73,549 B37979— 51,717	R34403— 70,364 R37136A— 55,596 R37136B— 81,417
Replication	Two technical replicates from each of two individuals	One technical replicate of ITGA6- enriched (I) or unsorted cells (T) from each of two individuals	Two technical replicates from each of two individuals	One technical replicate per sample derived from multiple pups in one litter	Two technical replicates from B36506, one technical replicate from B37979	Two technical replicates from R37136, one technical replicate from R34403
Accession #	GSE120508	GSE124263	GSE134144	GSE108970	GSE222105	GSE222105

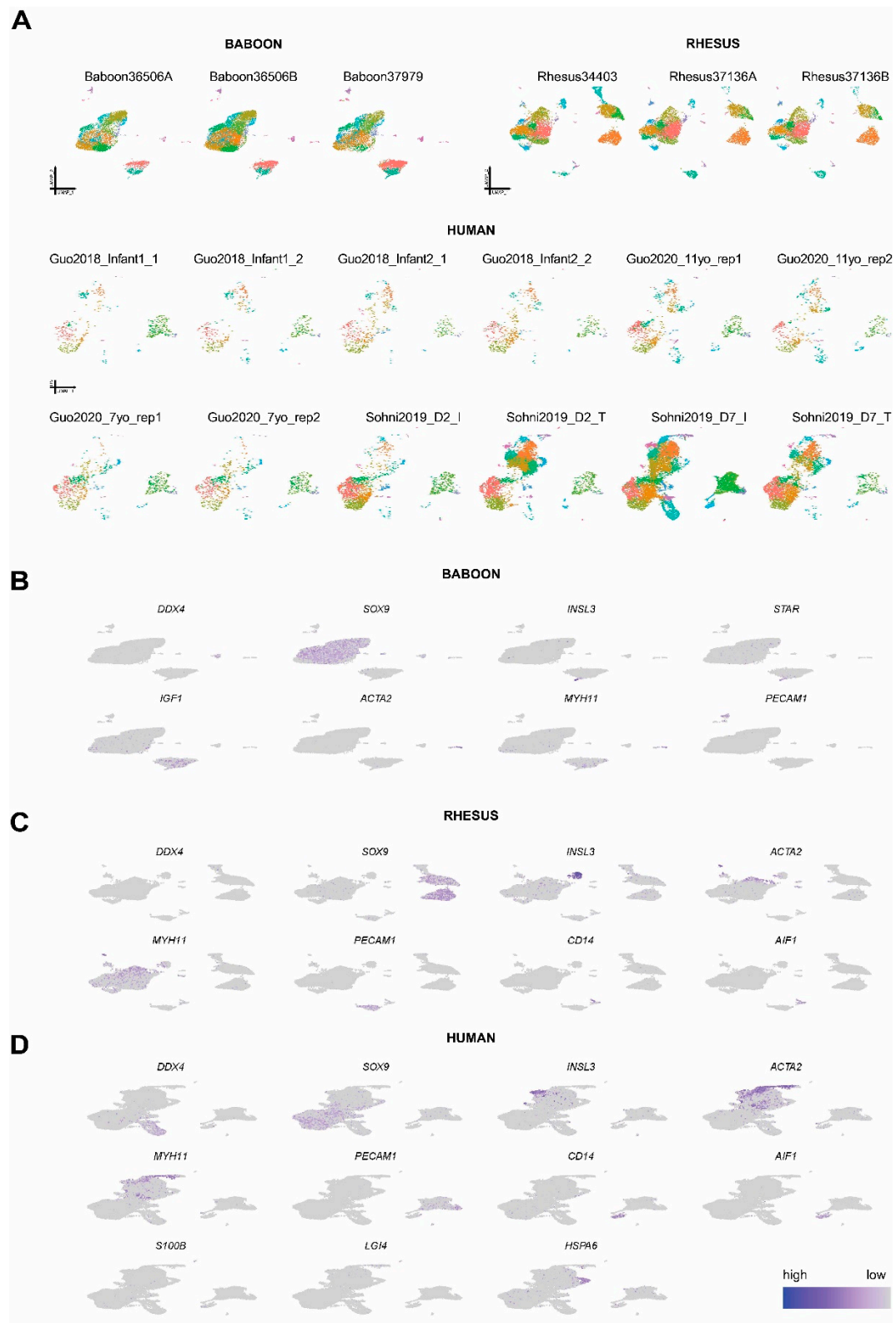


Figure S1. Profiling of single-cell transcriptomes of prepubertal human, baboon, and macaque testicular cells. UMAP plot visualization of the testicular cells from (A) baboon, rhesus macaque, and human displayed by replicates (same data as Figure 1). Each dot represents a single cell, which is colored by cluster annotations. (B–D) UMAP plots showing the testicular cells from (B) baboon, (C) rhesus macaque, and (D) human testes, which are color-coded to show the expression pattern of the selected gene markers specific for germ and somatic cell lineages.

Table S2. Differentially expressed genes and pathway analyses related to Figures 1–3. An Excel spreadsheet with multiple tabs relating to Figures 1–3. Each tab is labeled according to the relevant figure panel.

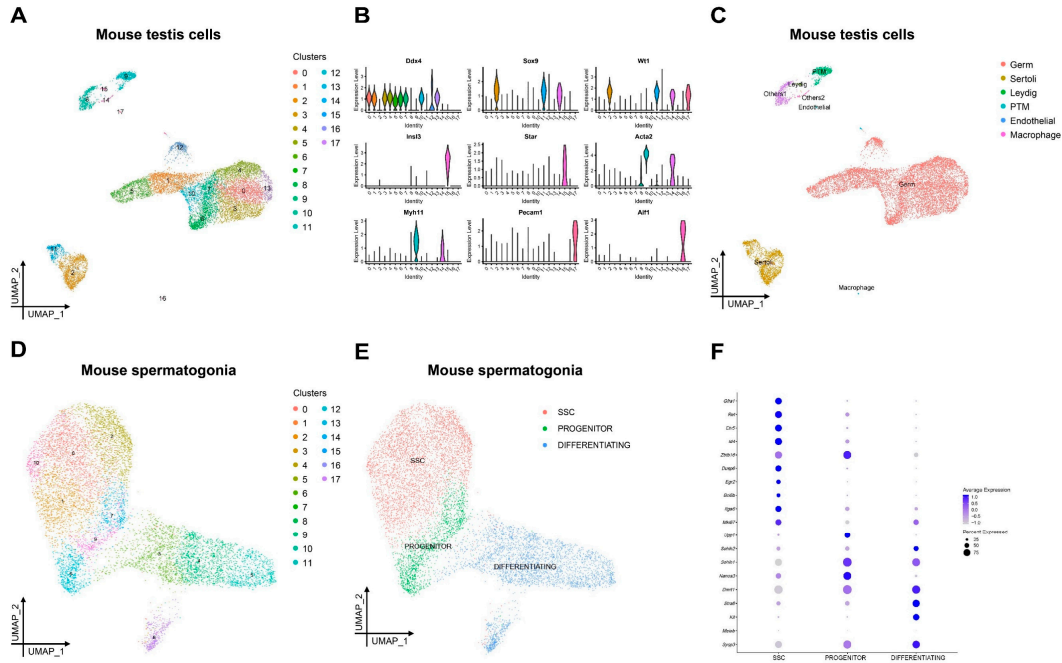


Figure S2. Single-cell transcriptome analysis of testis cells from postnatal day 6 (P6) mouse. (A and C) UMAP plot visualization of single-cell transcriptomes from P6 mouse testis ($n=13,554$) colored by (A) unbiased cluster annotation ($n=18$) and (C) colored by cell type. (B) Violin plots of mouse testes cells which are color-coded according to cluster annotations showing the expression of selected gene markers specific for germ and somatic cell lineages. (D-E) UMAP projections showing re-clustering of mouse germ cells colored by (D) unbiased clusters and (E) cell types. (F) Dotplot shows the expression of selected markers of undifferentiated and differentiated spermatogonia for each germ cell subset seen in UMAP plot labelled as SSC, progenitor and differentiating. The size of each dot represents the percentage of cells within a cell type, while the color encodes the average expression level across all cells within a cell type (blue is high).

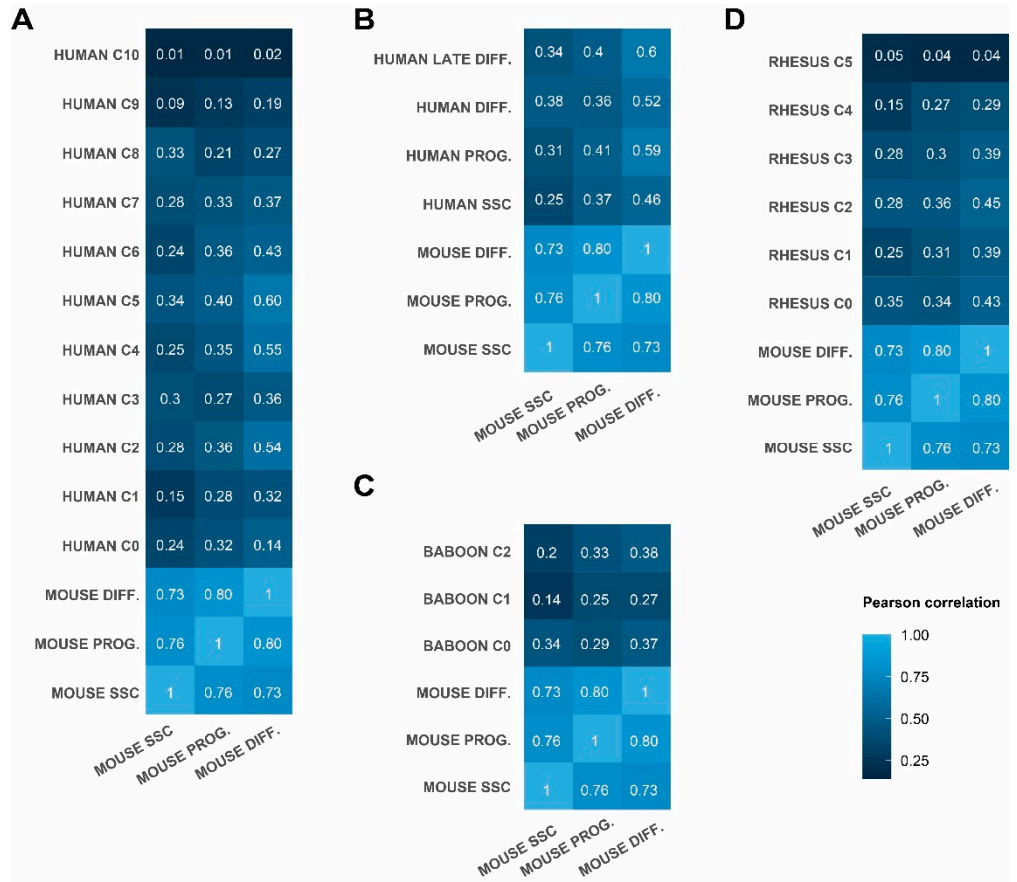


Figure S3. Cross-species comparison of mouse SSCs with human, baboon, and rhesus spermatogonial clusters. **(A)** Correlation heatmap shows the comparison of the mouse SSC cluster with human unbiased spermatogonial clusters (Human C0–10), colored based on the Pearson correlation coefficient. **(B)** Correlation heatmap shows the comparison of the mouse SSC cluster with human spermatogonial cell types (SSC, Progenitor, Differentiating, and Late differentiating). **(C)** Correlation heatmap shows the comparison of the mouse SSC cluster with unbiased baboon germ clusters (Baboon C0–2). **(D)** Correlation heatmap shows the comparison of the mouse SSC cluster with unbiased rhesus germ clusters (Rhesus C0–5).

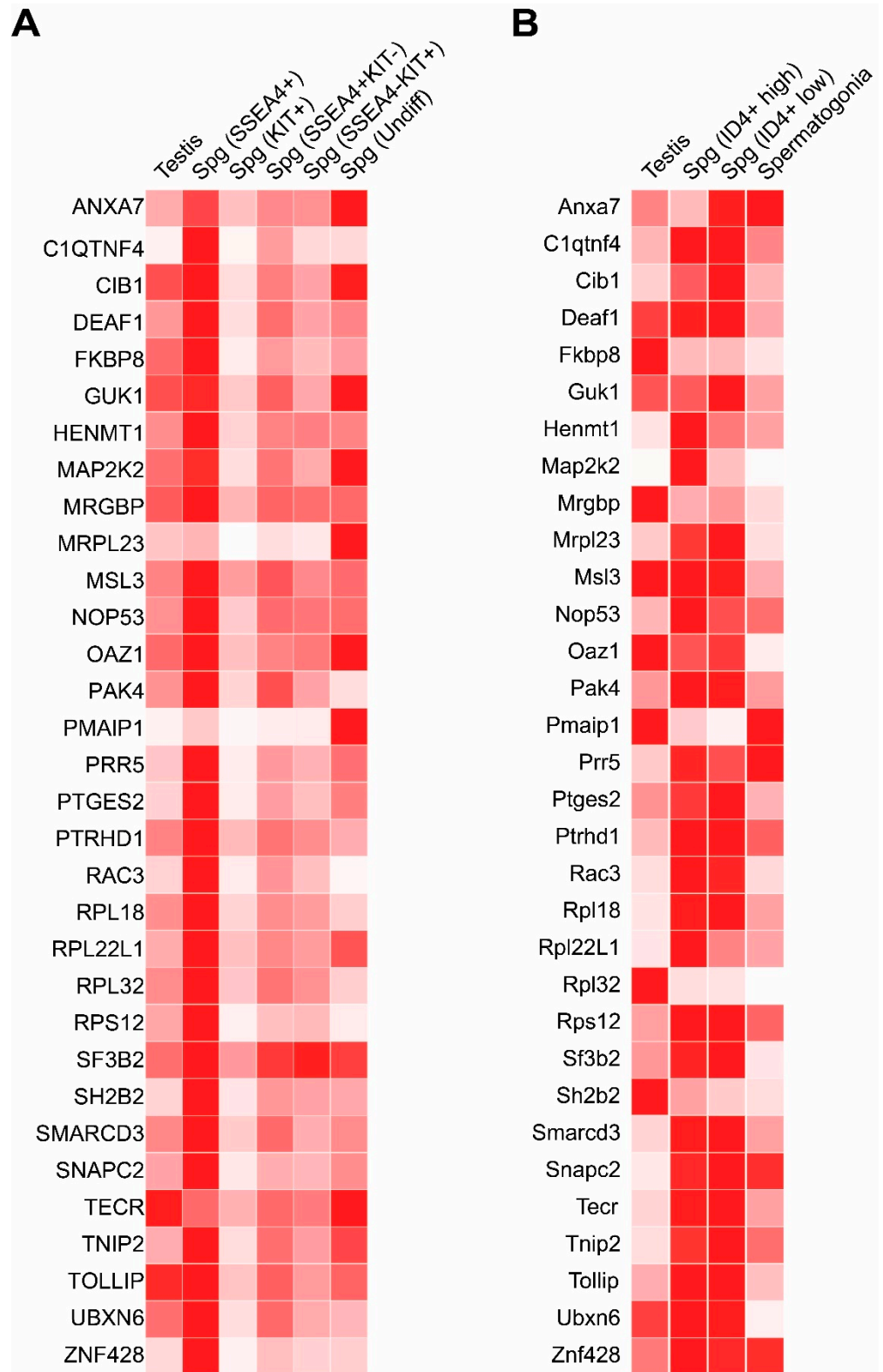


Figure S4. Primate SSC marker expression in published human and mouse RNA-seq datasets. Heatmap shows mRNA expression of 32 genes with elevated expression in the primate SSC clusters from our data in (A) human and (B) mouse spermatogonia from the Mammalian Reproductive Genetics Database (<https://orit.research.bcm.edu/MRGDv2>). Red denotes high abundance; white is low abundance. The columns are datasets and the rows are genes.