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Supplemental Information

**The Dynamic Transcriptional Cell Atlas
of Testis Development during Human Puberty**

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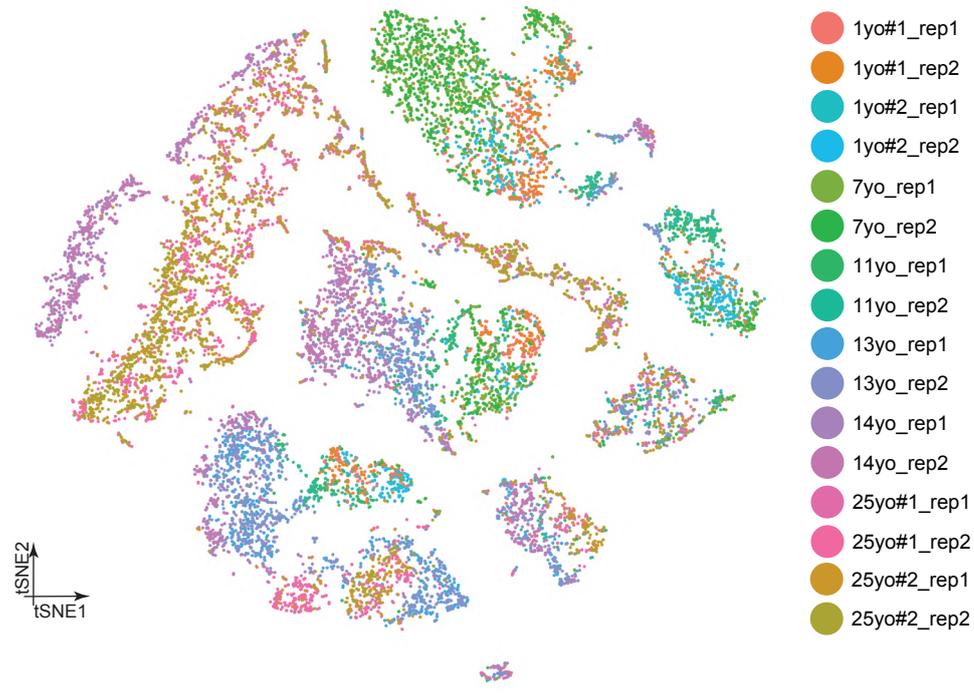
Supplemental Figures

Fig. S1

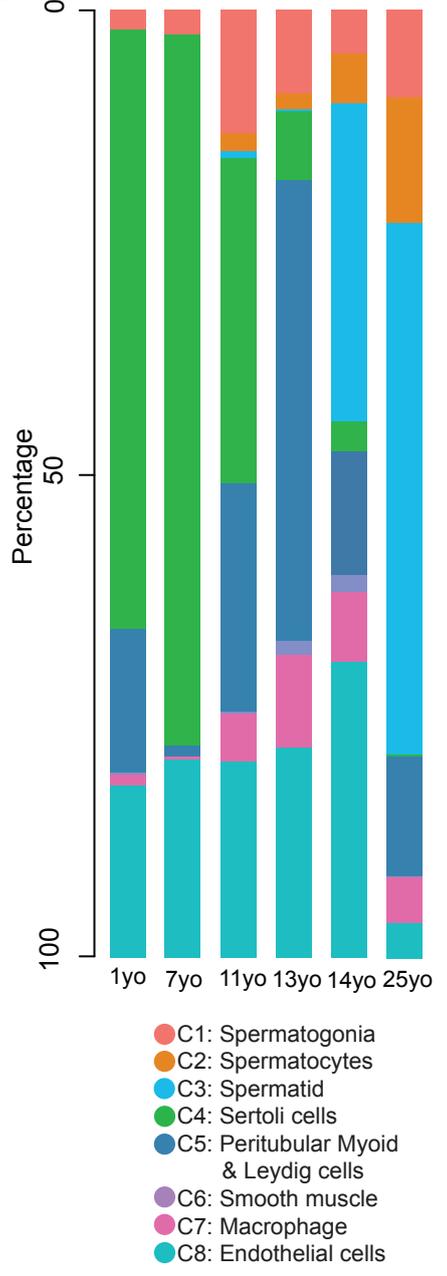
A

	Total reads	Mean reads/cell	Median gene/cell	Detected gene #	Captured cell #	Sequencing Saturation	Correlation
7yo Rep 1	210 M	203,705	1,612	20,932	1,030	93.4%	0.980
7yo Rep 2	291 M	310,094	1,644	20,868	938	95.5%	
11yo Rep 1	288 M	114,236	906	22,181	2,524	86.1%	0.964
11yo Rep 2	292 M	176,674	1,165	22,995	1,652	88.1%	
13yo Rep 1	263 M	125,116	1,482	24,514	2,104	84.5%	0.985
13yo Rep 2	280 M	143,635	1,684	24,510	1,947	84.8%	
14yo Rep 1	288 M	213,918	2,083	26,935	1,116	88.0%	0.994
14yo Rep 2	285 M	207,187	1,944	26,756	1,132	87.4%	

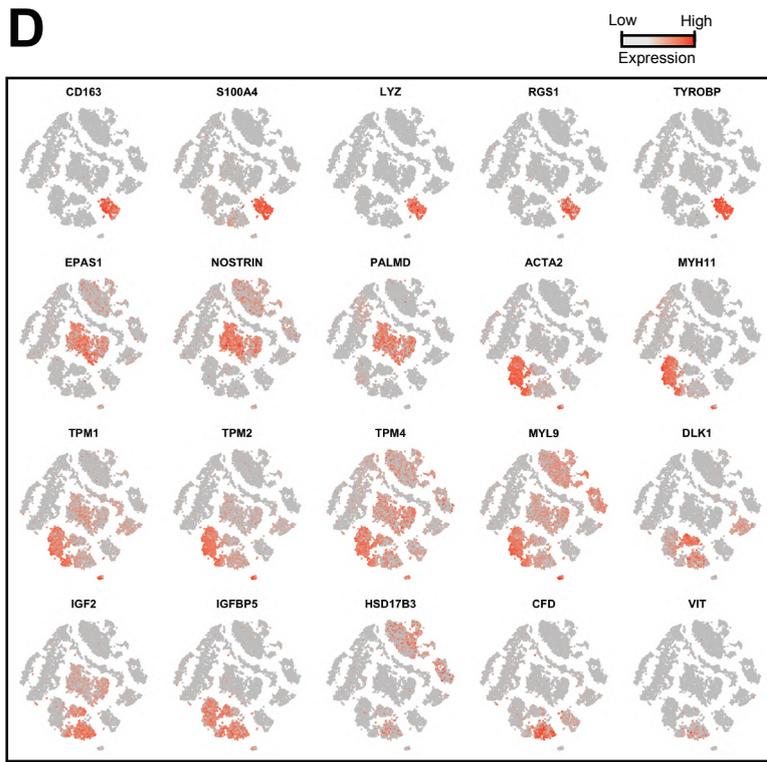
B



C



D



E

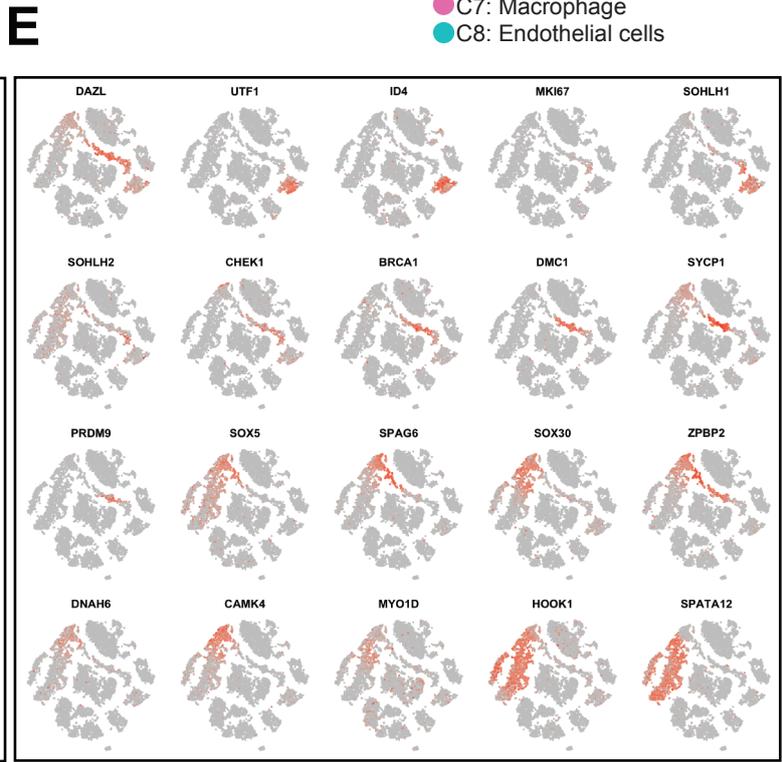


Figure S1. Quality control metrics of single-cell RNA-seq datasets

(A) Sequencing metrics of eight datasets (four juvenile samples; two technical replicates each sample) generated.

(B) tSNE plot of single-cell transcriptome with cells colored according to their donor of origin and replicate.

(C) Bar graph showing the relative proportion of different cell types/clusters for each sample/age.

(D) Gene expression patterns of additional markers of niche/somatic cells, projected onto the tSNE plot of Figure 1C.

(E) Gene expression pattern of additional markers in germ cells. Genes have been organized to mark more differentiated germ cell populations as they move from the top to the bottom rows.

Related to Figure 1.

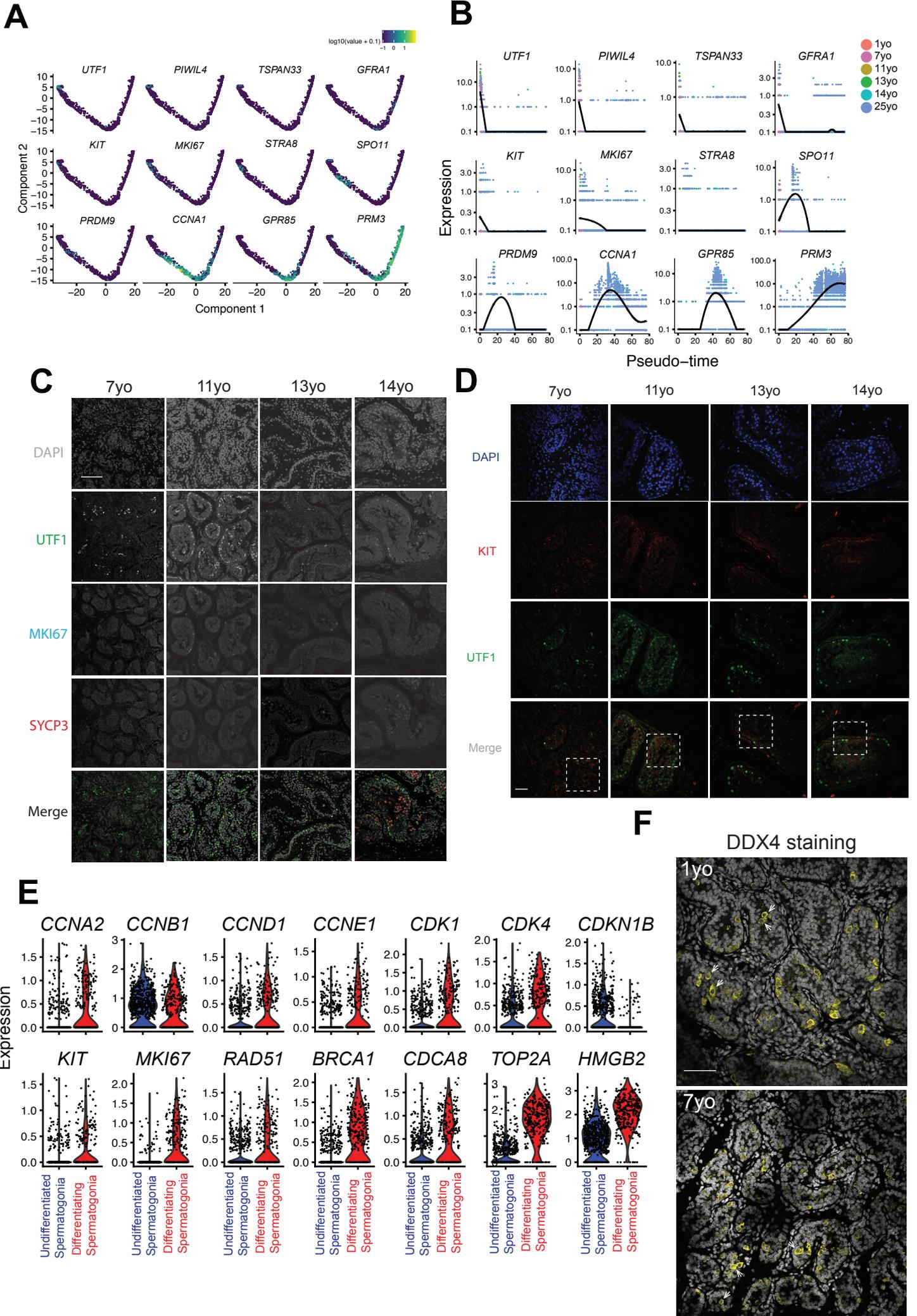
Fig. S2

Figure S2. Distinct phases of spermatogonial proliferation and differentiation during human puberty

(A) Expression patterns of representative markers projected onto the Monocle plot of Figure 2C. Key represents gene expression levels.

(B) Expression levels of representative dynamic genes during germ cell development, as predicted by pseudotime trajectory.

(C) Protein immunostaining of the samples analyzed (7-14yo) using three different germ cell markers: UTF1 is an undifferentiated spermatogonia marker (State 0-1), MKI67 expression overlaps with proliferating/differentiating spermatogonia, and SYCP3 is a meiotic marker. White bar represents = 100um.

(D) Low magnification of the FFPE section in Fig 2F. The boxed regions represent the part of the area shown at the higher magnification on Fig 2F. White bar represents = 20um.

(E) Violin plots of representative cell cycle related genes in undifferentiated and differentiating spermatogonia.

(F) IF staining for DDX4 in the pre-pubertal (1 and 7yo) testes. Although most germ cells (yellow) localize at the basement membrane, some germ cells do not (as indicated by white arrows). White bar represents = 100um.

Related to Figure 2.

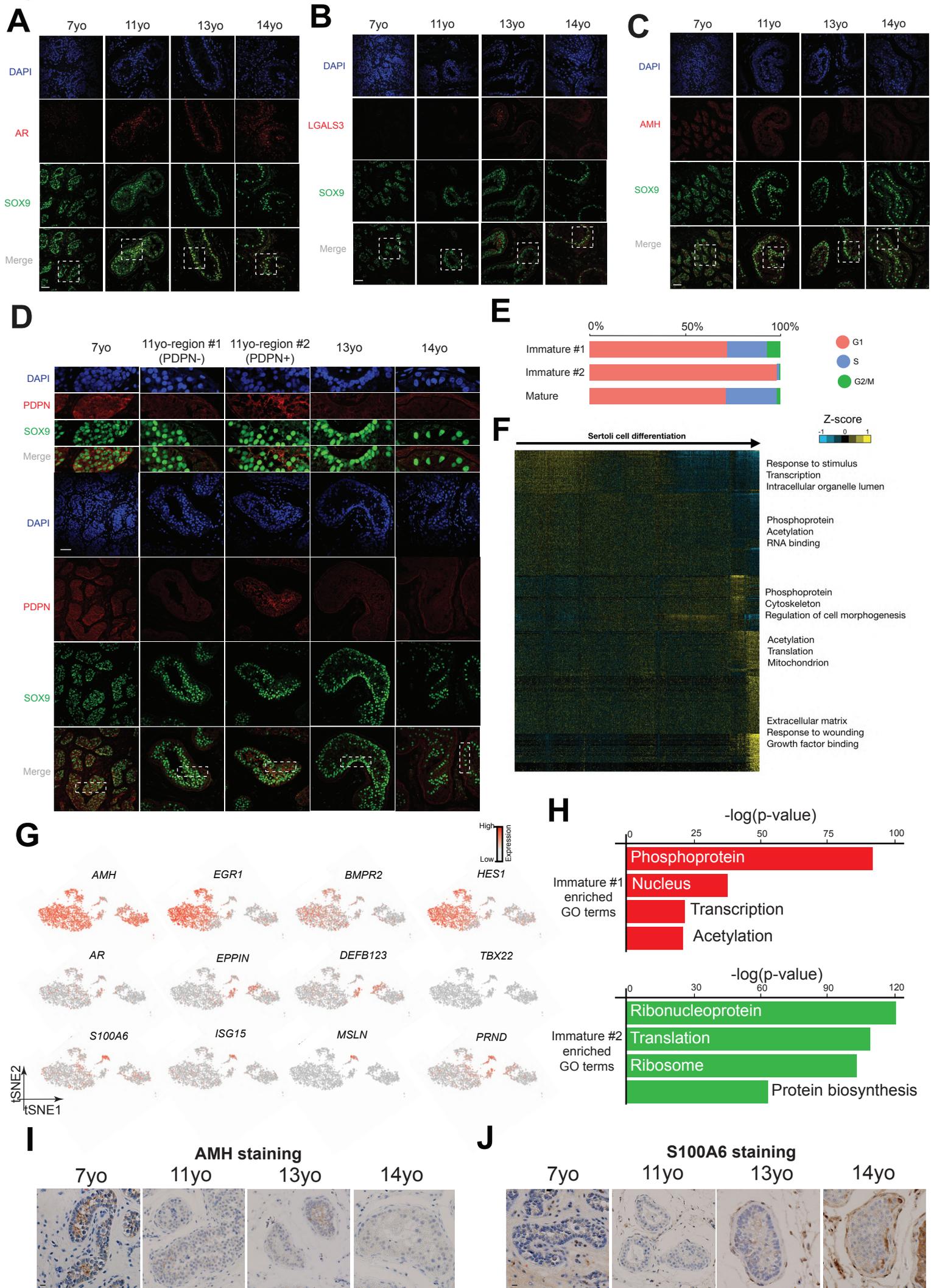
Fig. S3

Figure S3. Sertoli expansion and maturation during puberty

(A) Low magnification of the FFPE section in Fig 3H. The boxed regions represent the part of the area shown at the higher magnification on Fig 3H. White bar represents = 20um.

(B) Low magnification of the FFPE section in Fig 3I. The boxed regions represent the part of the area shown at the higher magnification on Fig 3I

(C) Low magnification of the FFPE section in Fig 3J. The boxed regions represent the part of the area shown at the higher magnification on Fig 3J.

(D) Immunofluorescent co-staining for SOX9 and PDPN at different ages (7-14yo) supports the gradual maturation of Sertoli cells over time observed by pseudotime trajectory.

(E) Deconvolution of the cell cycle phases (G1/S/G2) for the distinct populations of Sertoli cell States.

(F) K-means clustering of genes exhibiting differential expression ($n=1000$) during Sertoli cell differentiation/maturation. Note: each row represents a gene, and each column represents a single cell, with columns/cells placed in pseudo-developmental order. Differential gene expression levels utilize a Z score, which represents the variance from the mean, as defined on the color key in the right top corner, with associated GO terms (using DAVID v6.7) described on the right of the corresponding gene clusters.

(G) Expression patterns of representative markers projected onto the tSNE plot in Figure 3B.

(H) GO analysis of differential expressed programs that are associated with Immature Sertoli #1 (top) or Immature Sertoli #2 (bottom).

(I) Immunohistochemistry of the early Sertoli marker AMH at different ages (7-14yo). Black bar represents 20um.

(J) Immunohistochemistry of the later Sertoli marker S100A6 at different ages (7-14yo). Black bar represents 20um.

Related to Figure 3.

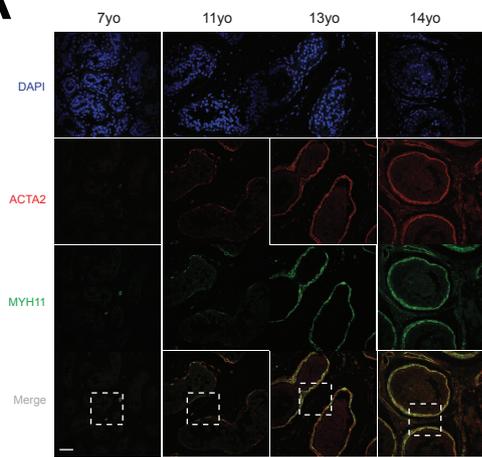
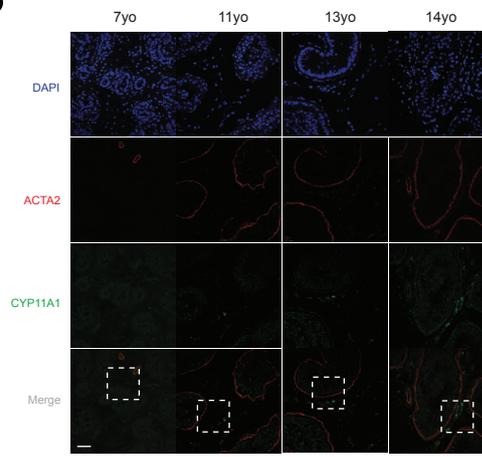
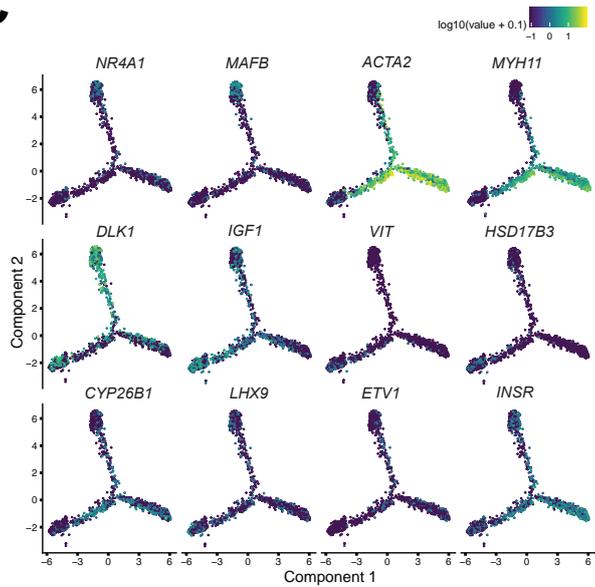
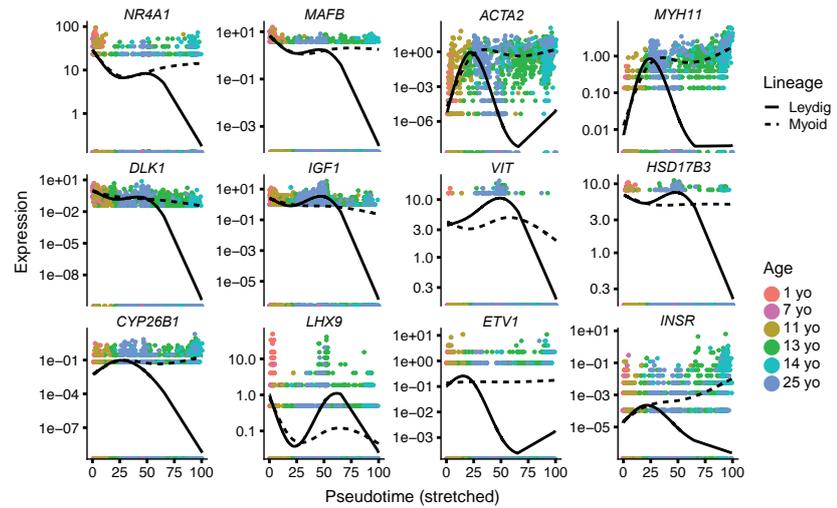
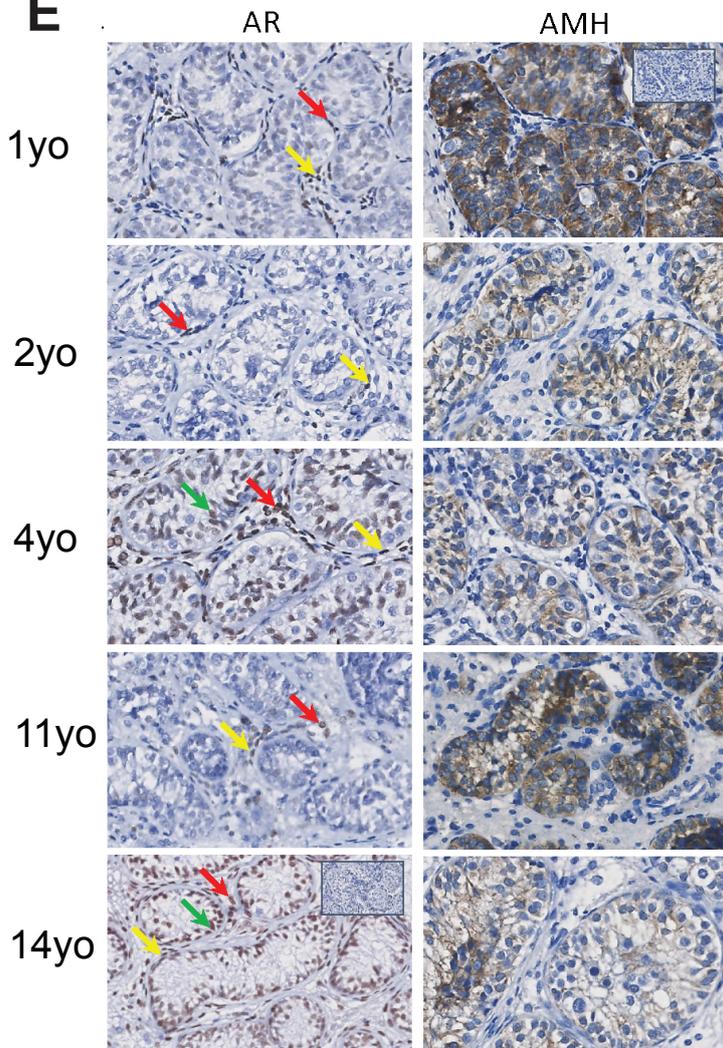
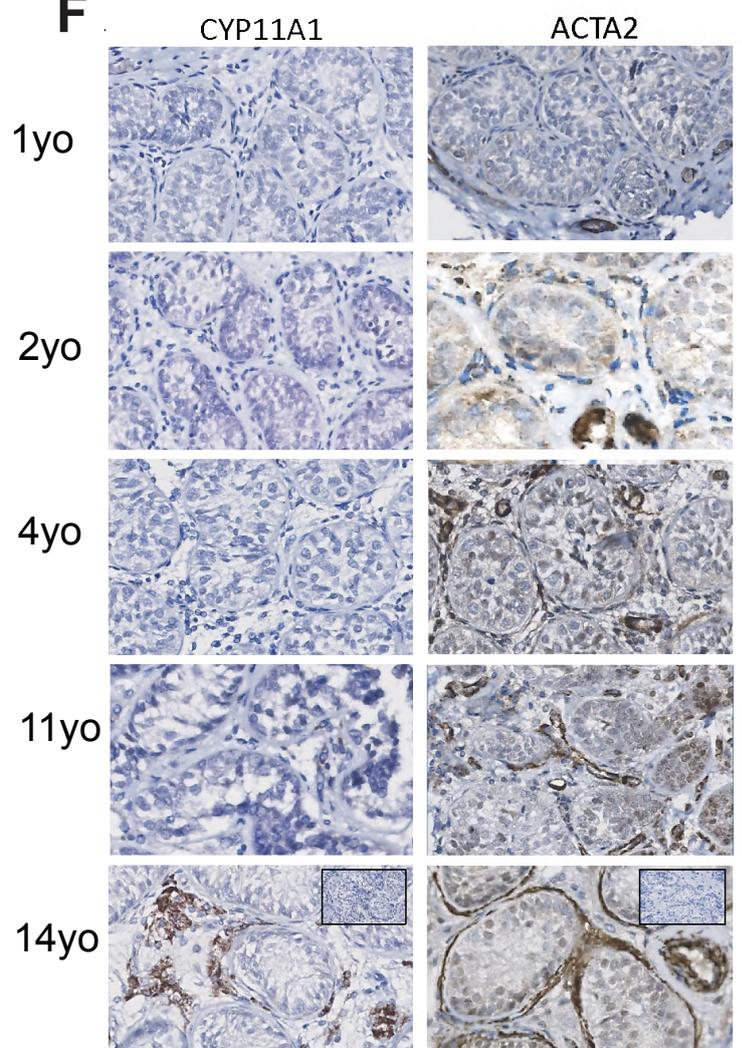
Fig. S4**A****B****C****D****E****F**

Figure S4. Leydig and myoid cells originate from a common precursor at prepuberty

(A) Low magnification of the FFPE section in Fig 4G. The boxed regions represent the part of the area shown at the higher magnification on Fig 4G. White bar represents = 20um.

(B) Low magnification of the FFPE section in Fig 4H. The boxed regions represent the part of the area shown at the higher magnification on Fig 4H.

(C) Expression patterns of representative markers projected onto the Monocle plot in Figure 4C

(D) Expression patterns of representative dynamic genes along Leydig (solid line) or myoid (dash line) lineage specification, as predicted by pseudotime.

(E-F) Orthogonal validation of genomics findings via additional juvenile testis samples. Immunohistochemistry staining of Sertoli cell markers (E) and Leydig/myoid markers (F) using additional juvenile samples. Yellow arrows: peritubular myoid cells; green arrows: Sertoli cells; red arrows: Interstitial (Leydig) cells.

Related to Figures 3 and 4.

Fig. S5

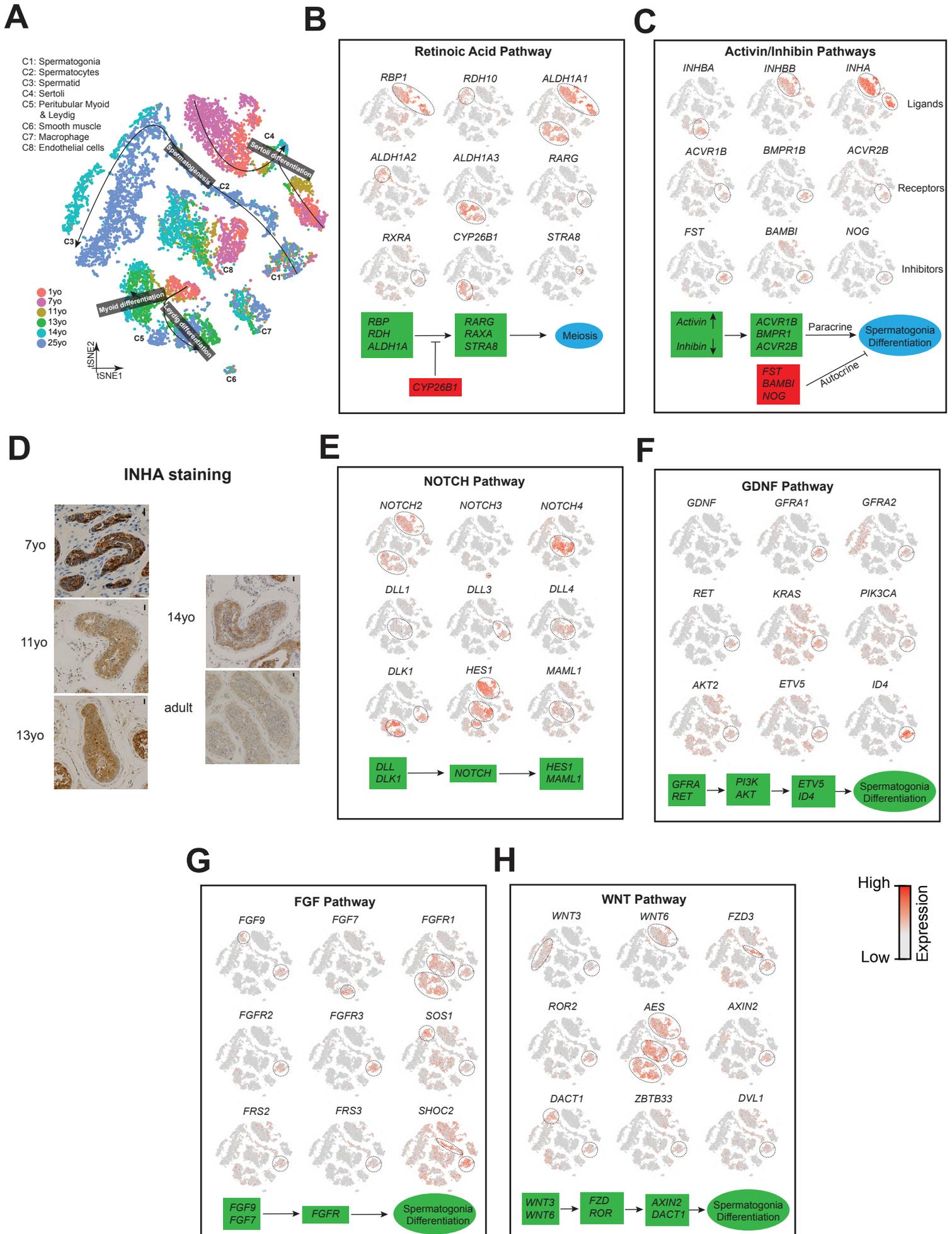


Figure S5. Overview of the key signaling pathways regulating testis development

(A) Summary of the tSNE plot of the combined single-cell transcriptome dataset, with the solid curve lines (with arrows) depicting the inferred developmental trajectories of the different cell clusters.

(B) Relative expression levels of representative genes from the retinoic acid (RA) pathway projected onto the tSNE plot from Figure S5A. Stage-specific expression is highlighted by dotted circles.

(C) Relative expression levels of representative genes from the Activin/Inhibin pathway projected onto the tSNE plot from Figure S5A. Stage-specific expression is highlighted by dotted circles.

(D) Immunohistochemistry on the analyzed samples shows that INHA expression (brown) decreases as puberty proceeds. Scale bar: 20um.

(E) Relative expression levels of representative genes within the NOTCH pathway projected onto the tSNE plot from Figure S5A. Stage-specific expression is highlighted by dotted circles.

(F) Relative expression levels of representative genes within the GDNF pathway projected onto the tSNE plot from Figure S5A. Stage-specific expression is highlighted by dotted circles.

(G) Relative expression levels of representative genes within the FGF pathway projected onto the tSNE plot from Figure S5A. Stage-specific expression is highlighted by dotted circles.

(H) Relative expression levels of representative genes within the WNT pathway projected onto the tSNE plot from Figure S5A. Stage-specific expression is highlighted by dotted circles.

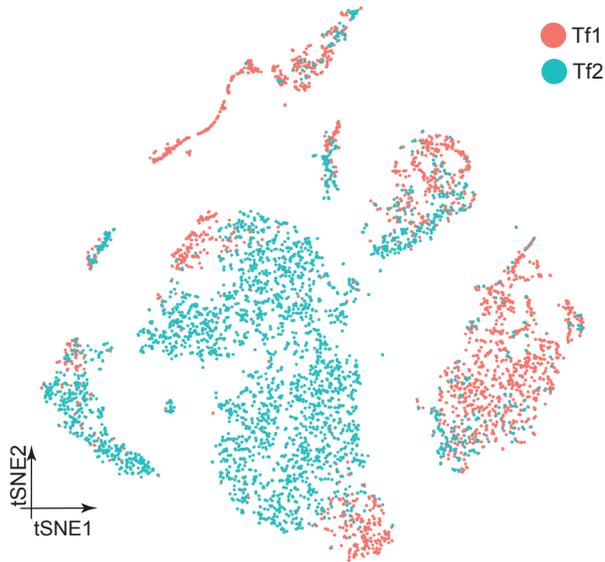
Related to Figures 3 and 4.

Fig. S6

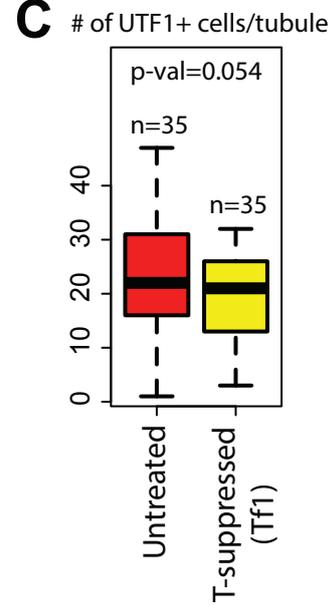
A

	Total reads	Mean reads/cell	Median gene/cell	Detected gene #	Captured cell #	Sequencing Saturation	Correlation
Transfemale #1 Rep 1	365 M	295,338	1,823	26,295	1,235	92.1%	0.978
Transfemale #1 Rep 2	434 M	287,884	1,877	26,412	1,509	93.2%	
Transfemale #2	234 M	58,884	1,282	22,683	4,036	83.1%	NA

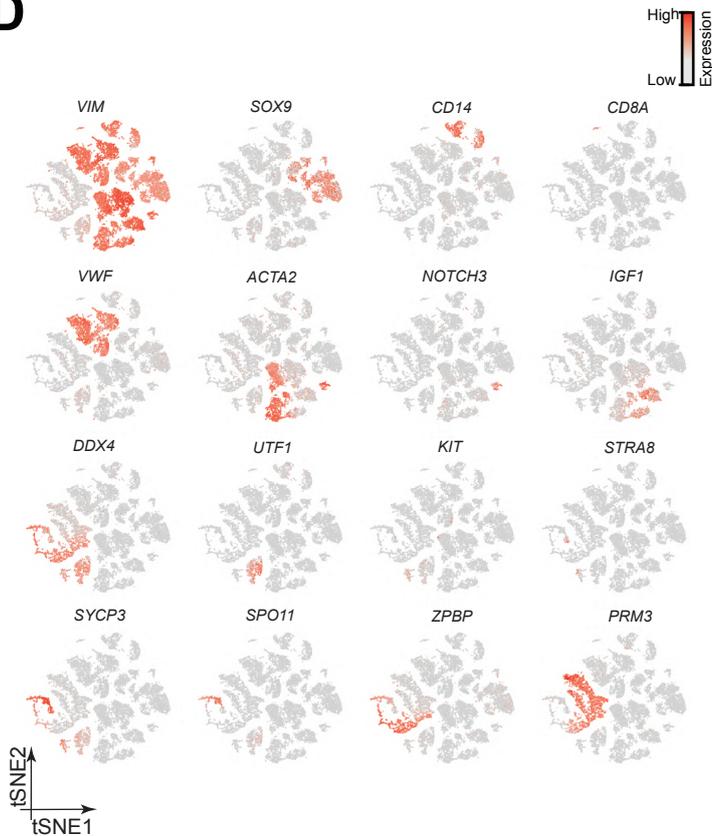
B



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D



E

Gene expression in Sertoli cells from untreated and T-suppressed testes

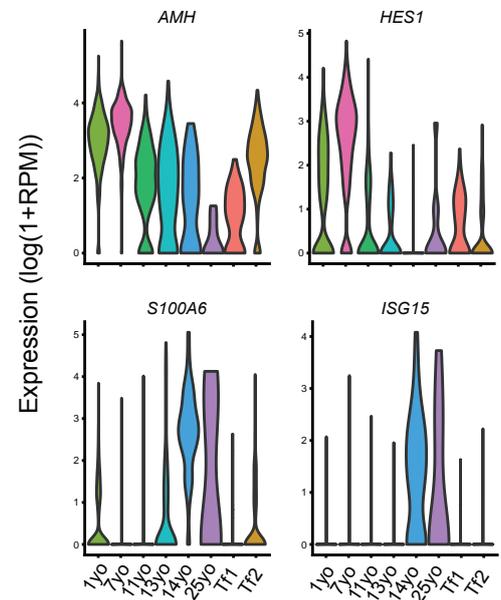


Figure S6. Single cell transcriptome profiling of testis from T-suppressed transfemales

(A) Sequencing metrics of three datasets generated.

(B) tSNE plot of single-cell transcriptome with cells colored based on their donor of origin.

(C) Quantification of number of UTF1+ quiescent spermatogonia (State 0-1) in untreated (25yo) and T-suppressed (Tf1) testis. No significant difference between the samples was observed ((Student t-test); p-value = 0.054).

(D) Expression patterns of selected markers projected onto the tSNE plot of Figure 6A (combined analysis of all untreated and T-suppressed samples). Top two rows represent somatic/niche cell markers; bottom two rows are representative germ cell markers.

(E) Violin plots showing the expression levels of representative Sertoli markers in T-suppressed and untreated at different ages in Sertoli cell populations.

Related to Figures 5 and 6.

Table S1: Summary of juvenile single-cell dataset and clustering information.
Related to Figure 1.

Table S2: Genes that show differential expression in two immature Sertoli States.
Related to Figure 3.

Table S3: Genes that show differential expression along Leydig-myoid cell development.
Related to Figure 4.

Table S4: Summary of transfemale single-cell dataset.
Related to Figure 5.