Supporting Information

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SI Materials and Methods

Isolation of round spermatids, pachytene spermatocytes, and spermatogonia plus early spermatocytes was performed by enzymatic dispersion and elutriation.

Reagents Used for Cell Isolation. ***Cell dispersion medium.*** DMEM/F-12 was supplemented with 1 mM sodium pyruvate and 13.4 mM sodium lactate.

Cell sedimentation medium. Cell dispersion medium was supplemented with 2% fraction V BSA.

Cell elutriation medium. Cell dispersion medium was supplemented with 1 mM EDTA, 0.5% fraction V BSA, and 50 units/ml heparin. Enzyme solution for isolation of pachytene spermatocytes and round spermatids was as follows: 0.1% collagenase (Worthington; CLS1), 0.2% hyaluronidase (Sigma; H6254), 0.034% DNase (Sigma; D5025), and 0.06% trypsin inhibitor (Sigma; T-9003). Enzyme solution for isolation of spermatogonia and early spermatocytes was as follows: 0.1% collagenase/dispase (Roche; 11 097 113 001), 0.2% hyaluronidase, 0.034% DNase, and 0.06% trypsin inhibitor.

Methods

Four testes from 55- to 75-day-old male Sprague–Dawley rats were used for each isolate of round spermatids and pachytene. Eighty testes from eighteen 20-day-old male Sprague–Dawley rats were used for each isolate of spermatogonia and early (primarily preleptotene) spermatocytes. This age of immature rat was selected based on the methods described by Bucci *et al.* (1) for the isolation by elutriation of spermatogonia plus early spermatocytes. Testes were decapsulated, blood vessels were removed, and the tissue was placed in a 50-ml plastic conical tube containing 20 ml of the appropriate enzyme solution (see above). The tube was submerged in a water bath set at 34°C and shaken at 80 oscillations per minute for 15 min. Tubule fragments were allowed to settle out of suspension, the medium with liberated cells was collected and centrifuged, and the cell pellets were resuspended in cell dispersion medium. The tubule fragments were resuspended in 20 ml of enzyme solution and incubated with shaking for an additional 15 min. Pipetting the tubules three times with a 10-ml pipette further dispersed the tubules, and tubule fragments then were allowed to settle out at unit gravity. Suspended cells and cell clumps in the supernatant were collected, and large cell clumps were removed by filtration through a 53-μm nylon mesh. The cells were then pelleted by centrifugation and suspended in 50 ml of cell sedimentation medium. The tube containing the cells was placed in a tissue culture incubator (34°C) for 20–30 min to allow clumps of Sertoli cells to settle out of the medium. The supernatant was collected, and the cells were pelleted by centrifugation and then resuspended in cell dispersion medium. The cells then were filtered sequentially through 30- and 20-μm nylon mesh, and the remaining Sertoli cell clumps allowed to settle out at unit gravity. The single cell suspension was pelleted by centrifugation and resuspended in elutriation medium. Round spermatids, pachytene spermatocytes, or spermatogonia plus early spermatocytes were isolated as previously described (2). The precise elutriation conditions are described in Table 1. During cell isolation, the elutriation chamber was maintained at 4°C and the cells were collected into 50 conical polypropylene tubes that were packed in ice. Cells in each tube were pelleted by centrifugation, resuspended in 2 ml of cell dispersion medium, and examined by phase-contrast microscopy to determine numbers and purity of the cells. Fractions containing round spermatids, pachytene spermatocytes, or spermatogonia plus early spermatocytes of at least 85% purity were collected. If this purity was not achieved, the cell preparation was discarded. Cells then were pelleted by centrifugation and solubilized in 1.2 ml of Buffer RLT with 1% 2-mercaptoethanol (Qiagen). The solubilized cells were then flash frozen by immersing the sample tubes in liquid nitrogen. The numbers of independent samples of each cell types were as follows: round spermatids ($n = 4$), pachytene spermatocytes ($n = 4$), and spermatogonia plus early spermatocytes ($n = 3$).

1. Bucci LR, Brock WA, Johnson TS, Meistrich ML (1986) Isolation and biochemical studies of enriched populations of spermatogonia and early primary spermatocytes from rat testes. *Biol Reprod* 34:195–206.

2. Shaper NL, Wright WW, Shaper JH (1990) Murine β1,4-galactosyltransferase: Both the amounts and structure of the mRNA are regulated during spermatogenesis. *Proc Natl Acad Sci USA* 87:791–795.

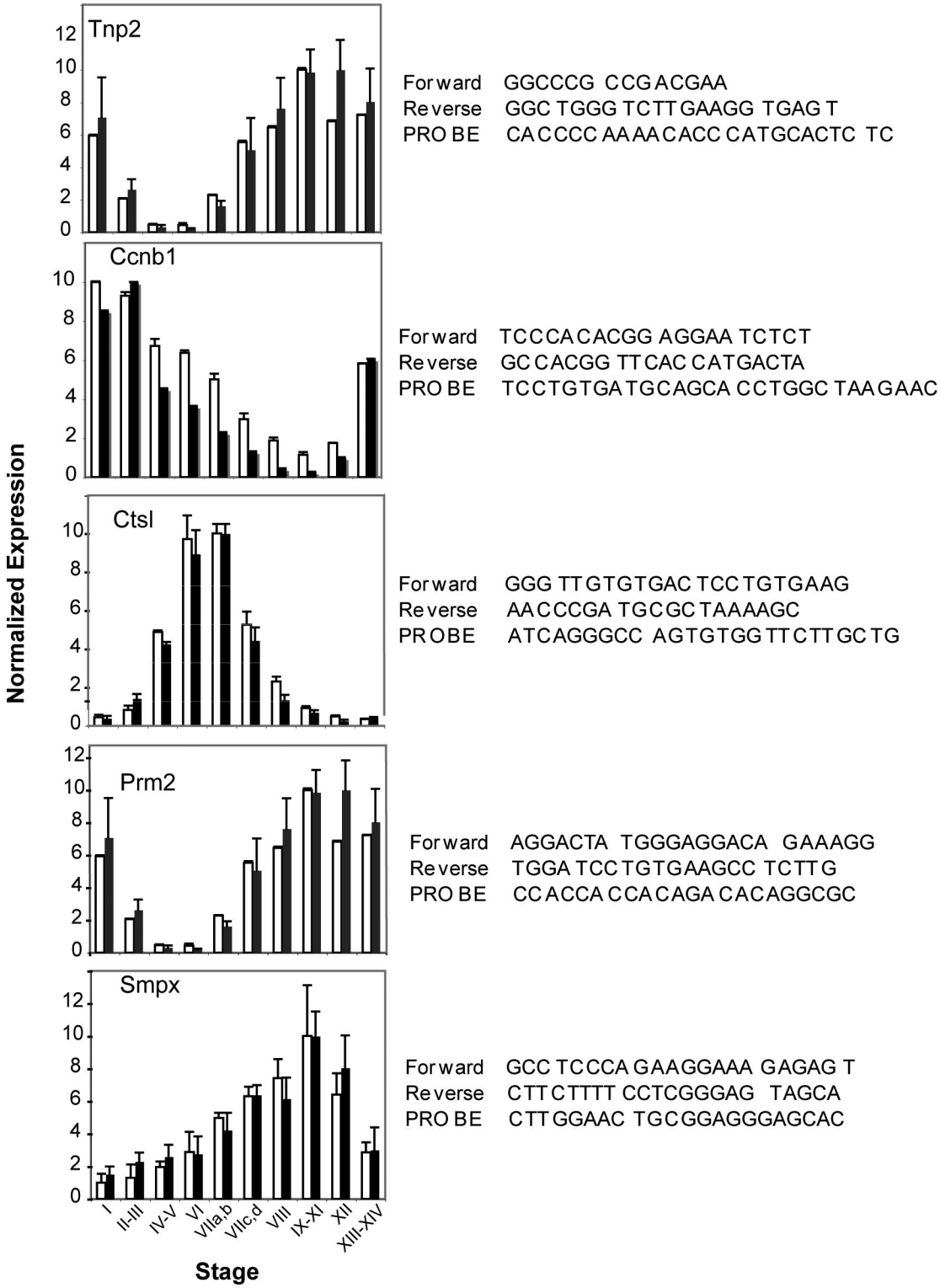

Fig. S1. Comparison of the measurement by Affymetrix array analysis and by quantitative real-time PCR of transcripts encoding transition protein 2 (Tnp2), cyclin B1 (Ccnb1), cathepsin L (Ctsl), protamine 2 (Prm2), and small muscle protein (Smpx). RNA was isolated from seminiferous tubules at defined stages of the cycle of the seminiferous epithelium, and this RNA was used in both assays. Four or five independent samples per stage were assayed by array analysis, and three independent samples were assayed by quantitative RT-PCR. The sequences of the TaqMan primers used for quantitative real-time PCR are shown next to the data for each gene. For purposes of graphical presentation, the mean expression of a gene at the stage of maximal expression was defined as 10 and expression at all other stages was normalized accordingly. The data are expressed as the means + SD.

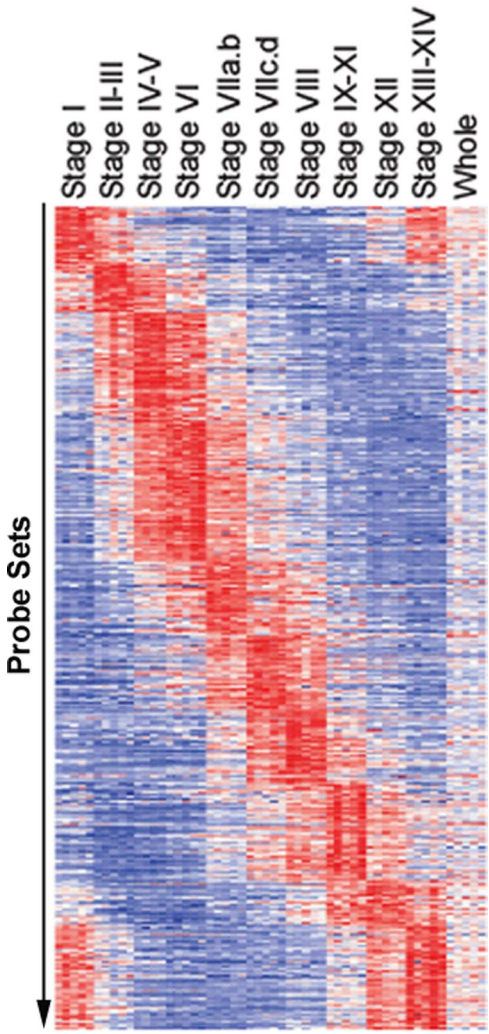

Fig. S2. The relative expression pattern of 1,612 probes sets differentially expressed at least 4-fold between at least two different stages of the cycle of the seminiferous epithelium. The data were obtained from 4 or 5 independent samples per stage or group of stages. Probe sets were ordered by the stage with the highest expression. Also shown are data obtained by analysis of whole testis. The data were visualized in Spotfire 8.0. Each probe set was *z*-scored normalized and color-coded (red, high expression; white, median expression; blue, low expression).

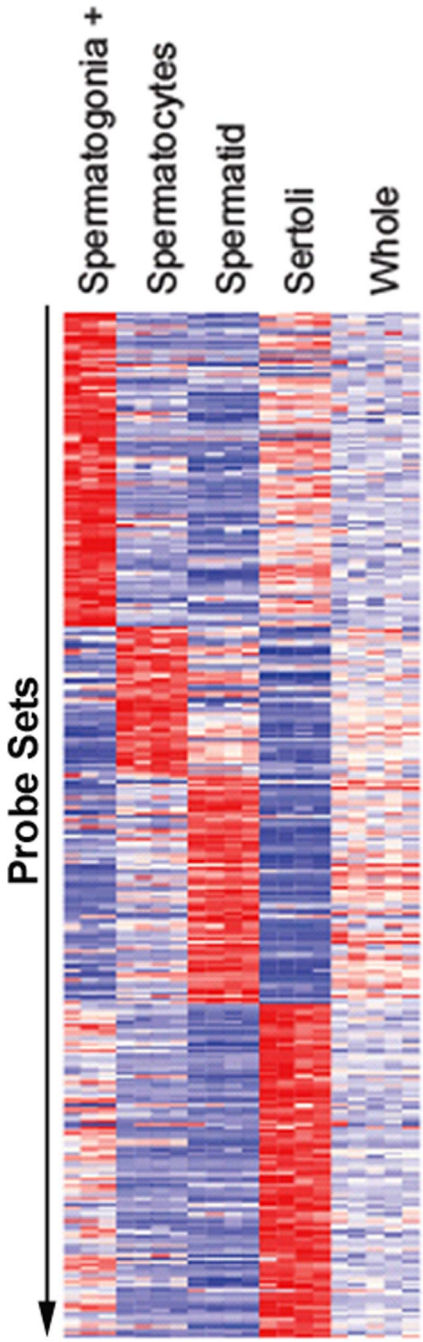

Fig. S3. The relative expression patterns of 9,550 probes sets differentially expressed at least 4-fold between at least two different testicular cell types. The data are shown for each sample of spermatogonia plus early spermatocytes (spermatogonia +) ($n = 3$), pachytene spermatocytes ($n = 4$), round spermatids ($n = 4$), Sertoli cells ($n = 4$), and total testis ($n = 5$). Probe sets were ordered by cell type with the highest expression. The data were visualized in Spotfire 8.0. Each probe set was z-scored, normalized, and color-coded (red, high expression; white, median expression; blue, low expression).

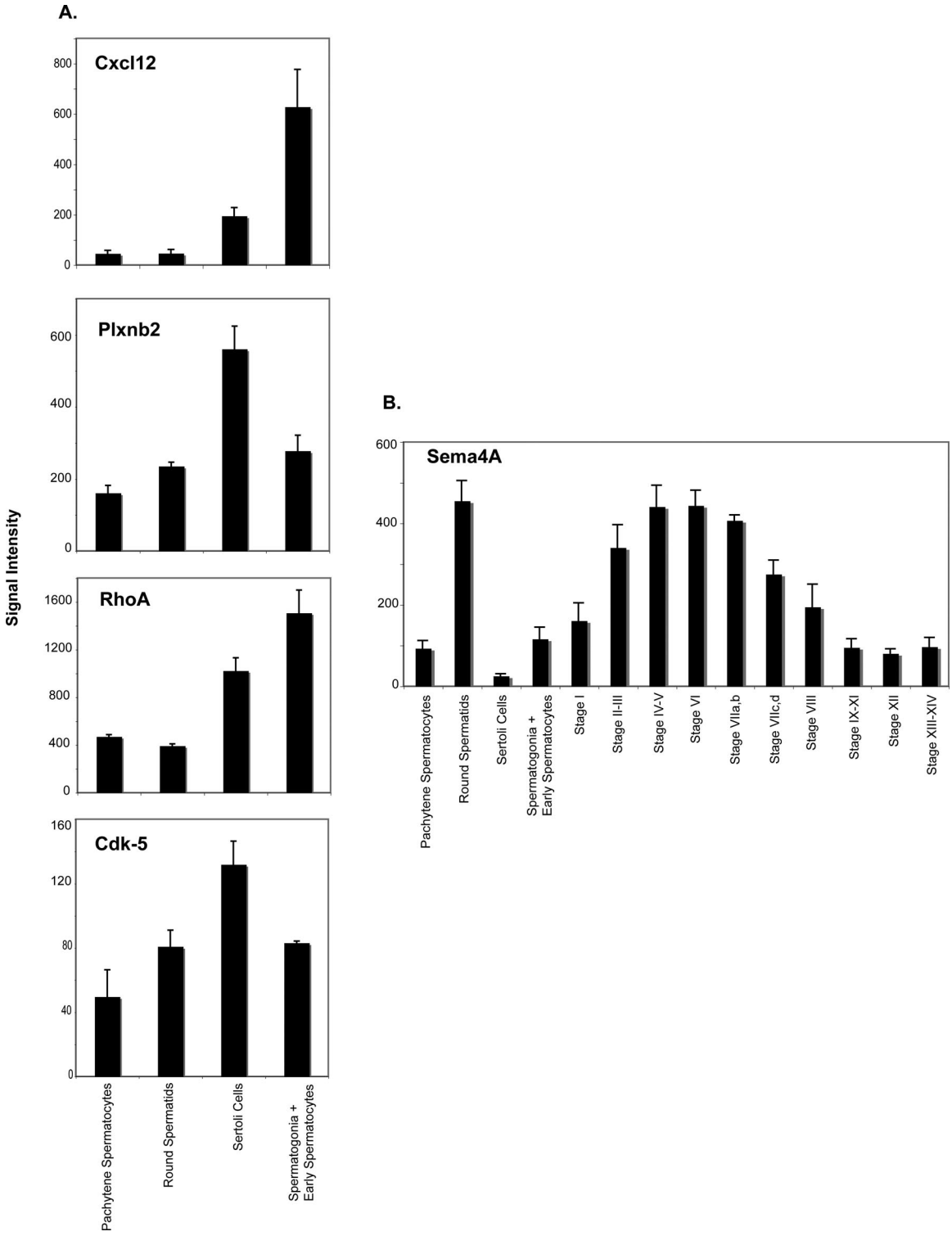

Fig. S4. Transcriptional expression by Sertoli cells and by spermatogenic cells of additional genes previously shown to regulate embryonic neuron development. (*A*) Expression by pachytene spermatocytes, round spermatids, Sertoli cells, and spermatogonia plus early spermatocytes of Stromal cell-derived factor 1/chemokine (C-X-C motif) ligand 12 (Cxcl12), plexin B2 (Plxnb2), RhoA, and cyclin-dependent kinase-5 (Cdk-5). (*B*) Expression by pachytene spermatocytes, round spermatids, Sertoli cells, spermatogonia plus early spermatocytes, and seminiferous tubules at defined stages of the cycle of the cycle of the seminiferous epithelium of Semaphorin 4a (Sema4a). The data (means + SD) are expressed as signal intensities from the array.

Table S1. Elutriation conditions for isolation of round spermatids, pachytene spermatocytes, and spermatogonia plus early spermatocytes

Tube no.	Rotor speed, rpm	Flow rate, ml/min
Round spermatids and pachytene spermatocytes		
A 1–6	2,000	26
B 7, 8	2,000	31
C 9, 10	2,000	36
D 11, 12	2,000	45
E 13, 14	0	45
F 15–20	2,500	13
G 21, 22	2,500	25.25
H 23, 24	2,500	31
I 25, 26	0	31
Spermatogonia and early spermatocytes		
A 1–6	2,500	13
B 7, 8	2,500	25
C 9, 10	2,500	37
D 11, 12	2,500	50
E 13, 14	0	50

During isolation of round spermatids and pachytene spermatocytes, after samples E13, 14 were collected, cells in tubes A 1–6 were reapplied to the elutriator with the rotor speed set at 2,500 rpm.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)
Dataset S5 (XLS)
Dataset S6 (XLS)