## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Molecular cloning and lentiviral infection

Dox-inducible embryonic Sertoli factors were generated by cloning the open reading frame of the factors, obtained by reverse transcription with specific primers (see Extended Experimental Procedures), into the TOPO-TA vector (Invitrogen), and then restricted with *Eco*RI or MfeI and inserted into the FUW-teto expressing vector. Replication-incompetent lentiviruses containing the embryonic Sertoli factors were packaged in 293T cells and collected after 48, 60 and 72 hr after transfection. The supernatants were filtered through a 0.45μm filter, supplemented with 2μg/ml of polybrene and then used to infect MEFs, TTFs or Keratinocytes.

### Cell proliferation and migration assays

Cells were seeded in 6-wells plates at 40% confluence and let grow for 48 hours. Cell proliferation was determined by using the APC BrdU staining flow kit (BD Biosciences), following the manufacturer's instructions. HUVEC and ieSCs migratory potential was assayed using the Boyden Chamber (8 μm membrane) based cell migration assay kit (Millipore) according to the manufacturer's instructions. Briefly, $3X10^5$ H2b-GFP-MEFs, H2b-GFP-ieSCs or H2b-GFP-immature Sertoli cells were seeded on top of a microporous membrane that contained a serum-free medium in the upper part and 10% FBS medium in the lower part. After 24 hours the number of the cells was analyzed by counting the GFP-positive cells that had migrated through the membrane. Cell motility was assayed with/without serum for 24 hr with Nikon Eclipse TE2000-U microscope. Images were acquired with a time lapse of 5 min.

**Western blot analysis**

Cells were lysed in buffer (10% SDS, 0.1M DTT, 10% glycerol, 1M Tris and Urea) supplemented with protease inhibitor cocktail (Roche) for 30 minutes on ice. Extracts were analyzed for protein concentration by BCA kit (Thermo scientific). The following primary antibodies (1:500 dilution) were used: anti-Cdkn2a$^{p19ARF}$ (ab80, Abcam), anti-Cdkn2a$^{p16INK4A}$ (F-4, Santa cruz biotechnology), anti-Cdkn2b (AB-74647, Abcam) and anti-Gapdh (G9545, Sigma 1:3000 dilution). The protein-antibody complexes were detected using horseradish peroxidase–conjugated secondary antibodies (CalBioChem) and the SuperSignal enhanced chemiluminescence system (Pierce).

**Immunofluorescence**

Cells were fixed in 4% paraformaldehyde in PBS for 20 min, rinsed 3 × with PBS, blocked for 1 hr with PBS containing 0.1% Triton X-100 and 5% FBS, and incubated O/N in PBS containing 0.1% Triton X-100 and 1% FBS with one of the following antibodies (1:100 dilution): anti-Nr5a1 (H-60, Santa cruz biotechnology), anti-Wt1 (C-19, Santa cruz biotechnology), anti-Dmrt1 (H-240, Santa cruz biotechnology), anti-Gata4 (H-112, Santa cruz biotechnology), anti-Sox9 (H-90, Santa cruz biotechnology), anti-Dsp (H-300, Santa cruz biotechnology) , anti-Cdh1 (H-108, Santa cruz biotechnology), anti-Amh (B-11, Santa cruz biotechnology), anti-Vim (AB28028, Abcam), anti-Lama1 (ab30320, Abcam) and anti-Col4a (AB756PMI, Millipore). Then, the cells were washed 3 × with PBS, incubated in PBS containing 0.1% Triton X-100 and 1% FBS with the relevant (Alexa) secondary antibody (1:200 dilution) for 1 hr together with DAPI (1:1000). The cells were washed 3 × with PBS and visualized under a fluorescence microscope (Nikon eclipse Ti-U).

**Cytogenetic analysis**

ieSCs were collected after one year of culturing and 17 passages and analyzed for metaphase spreads. The cells were prepared and analyzed for numerical and structural abnormalities as described (Babu et al., 2003).

***In vivo* tubulogenesis assay and Immunohistochemistry**

Pellet of cells that contained H2b-GFP ieSCs together with cells from 7 day-old mouse immature testis (one pellet contains $2X10^6$ ieSCs per one testis) was transplanted subcutaneously into NOD/SCID mice using small incision. Three weeks post-injection the transplants were removed, fixed in 4% PFA over night and embedded into paraffin for immunhistochemistry staining. The paraffin was removed by successive rinsing of slides for 3x2 minutes with xylene, EtOH 100%, 95%, and H2O. Antigen retrieval was maximized by heating the slides in 50 mM sodium citrate pH 6.0 in a microwave oven for 4x6 min and 10 min wash in Methanol. The slides were then blocked with 2% BSA for 30 min and stained over night with 1:5000 GFP antibody (ab290, Abcam) in 0.1% triton PBS solution at 4°C. After rinsing in PBS, the slides were blocked with 2% BSA and incubated with avidin-biotin complex solution for 30 min. Then the sections were washed in PBS and incubated in DAB solution.

**qRT-PCR Primers**

| Primer name | Forward | Reverse |
|---|---|---|
| Adm | ACACTGCAGGGCCAGATACT | AGTTCCCTCTTCCCACGACT |
| Aldh1a1 | CACTGGCCGACTTGAAGATT | TTTCTTGCCGCTCACTGAAT |
| Amh | CCACGGTTAGCACCAAATAGC | CACACAGAACCTCTGCCCTACTC |
| Bglap | CAGCGGCCCTGAGTCTGA | TTATTGCCCTCCTGCTTGGA |
| Cdh1 | AGCTGCCCCGAAAATGAAA | GATCTGAACCAGGTTCTTTGGAAA |
| Cdh2 | GGCGTCTGTGGAGGCTTCT | GGAAATCCAGTCTTGCATAATGC |
| Cdkn1a | ACATCTCAGGGCCGAAAA | TCTCTTGCAGAAGACCAATCTG |
| Cdkn2a | GAACTCTTTCGGTCGTACCC | TGAGCAGAAGAGCTGCTACG |
| Cdkn2b | AGATCCCAACGCCCTGAAC | CAGCAGCTCTGCCACCTG |
| Cgn | ACCCGAAAAATGGAGGAACT | CAACCCTGGATGGTTCTAGC |

| | | |
|---|---|---|
| Cited1 | GAGGCCTGCACTTGATGTC | CAAGGTTGGAGTAGGCCAGA |
| Clu | TGAAGGGCCAGTGTGAAAAGT | TTGAACAGTCCACAGACAAGATCTC |
| Col5a2 | TAGAGGAAGAAAGGGACAAAAAGG | GTTACAACAGGCACTAATCCTGGTT |
| Col9a1 | CCTGGGTATCCGCAACTCT | GGACACAGTTCACTTCCACCA |
| Cyp11a1 | TCCATTACCATCAGATGCAGA | GGGGTCCACGATGTAAACTG |
| Cyp26b1 | AAGGGCTCCATGGGATTC | ACGACTGGAAGCCGGAAC |
| Dclk1 | CAAGTCCATCACCCACCAG | GGATGAAAGTGAAGTCGAGGA |
| Dhcr7 | AGGCTGGATCTCAAGGACAA | GAGCAGCAGCAGGAAAATGATGC |
| Dhh | CCGCAACCACATCCACGTA | CGGACCGCCAGTGAGTTATC |
| Dmrt1 | GGAGTCTCCCAGCACCTTACG | TCTGCCACTGGTTTCCAGTCT |
| Dmrt1endo | GGTTGTAACCAAGTTTTCAGGA | CCGCTCTTCTCACTGGTCA |
| Dsp | ACCGTCAACGACCAGAACTC | TTTGCAGCATTTCTTGGATG |
| Erbb4 | CAGCGCTTCTCAGTCAGTGT | CTGCTGTTCCAGGTCAGAGA |
| Fgf9 | TCTTCCCCAACGGTACTATCCA | CGAAGCGGCTGTGGTCTTT |
| Fn1 | AGAGGCAGGCTCAGCAAAT | TGCTTCCCATTGTCAAAACA |
| Foxc2 | AGAACAGCATCCGCCACAAC | GCACTTTCACGAAGCACTCATT |
| Gata4 | CCCCAATCTCGATATGTTTGATG | TTGACACACTCTCTGCCTTCTGA |
| Gdnf | GGGTGCGTTTTAACTGCCATA | GCCCAAACCCAAGTCAGTGA |
| Gsc | GTCAGAAAACGCCGAGAAGTG | TCCGGCGAGGCTTTTGA |
| Hprt | GCAGTACAGCCCCAAAATGG | GGTCCTTTTCACCAGCAAGCT |
| Hsd3b1 | TGGACAAAGTATTCCGACCAG | TTCCAACACTGTCACCTTGG |
| Hsd3b6 | TGGACAAGTTCTTCAGACCAGA | TCTCCTTCCAACACTGTCACC |
| Irx3 | AGCCGGAGAGTGGAACAGAT | TGGAAAGCTGTCTTGAGTAACTTTT |
| Kitl | TGGTGGCAAATCTTCCAAATG | CGGCGACATAGTTGAGGGTTAT |
| Krt18 | GATTGAGGAGAGTACCACAGTTGTCA | TCCCTGATTTCGGCAGACTT |
| Lef1 | TCTTTGGTTAACGAGTCCGAAATC | CACCTCATGCCCGTTGCT |
| Mmp3 | ATCCCACATCACCTACAGGATTG | TGTCTTGGCAAATCCGGTGT |
| Muc1 | TACCAAGCGTAGCCCCTATG | CACCACAGCTGGGTTGGTAT |
| Nr5a1 | CCTCGATGTGAAATTCCTGAACA | TCCTGGGCGTCCTTTACG |
| Nr5a1endo | AGAGAAGTGGGCAGGAGACA | GCTTTGATGCTAGTCCCCATA |
| Ocln | GGCCTTTTGAAAGTCCACCT | CATGTCATTGCTTGGTGCAT |
| Postn | ACAACAATCTGGGGCTTTTT | AATCTGGTTCCCATGGATGA |
| Ptgds | GGCTCCTGGACACTACACCT | CATAGTTGGCCTCCACCACT |
| Runx2 | TCAAGGTTGTAGCCCTCGGA | TGACGGTAACCACAGTCCCAT |
| S100a4 | CCTCTCTATTCAGCACTTCCTCTC | GCCTCCTCCAAGGGTCTT |
| Shbg | CCAAAATCAGCAAACCCCATT | CTGGATCCCAGGTTCGAAAC |
| Snai1 | TGCTGACCGCTCCAACCT | CTTCACATCCGAGTGGGTTTG |
| Snai2 | ATCCTCACCTCGGGAGCATA | TGCCGACGATGTCCATACAG |
| Sox9 | GCATCTGCACAACGCGG | AGCCTCCAGAGCTTGCCC |
| Tdrd6 | GCCAGAGAAGGAAGGAAGTG | TTTCGATCCGACAGAGAAGC |
| Thy1 | CGAATCCCATGAGCTCCAAT | CCAGCTTGTCTCTATACACACTGATA |
| Twist1 | ACGCTGCCCTCGGACAA | CCTGGCCGCCAGTTTG |
| Vim | AACTGCACGATGAAGAGATCCA | GGACATGCTGTTCCTGAATCTG |
| Vnn1 | GGAAGGAGCGATCGTATCTG | CCGTATATGCCATCTTCTGGA |
| Wt1 | TTGAATGCATGACCTGGAATCA | TTCCCTTTAAGGTAGCTCCTAGGTT |
| Wt1endo | CCTCCTCTTGTGCAGGATGTG | AGATGCCCCAGATGATGGAA |

**Primers for the cloning of the ORF of the nine embryonic Sertoli factors**

| Primer name | Forward | Reverse |
|---|---|---|
| Dmrt1cDNA | GGAGACGAAGCTCCATGC | ACAAACCCTGCCGATAACAA |
| Gata1cDNA | CCAGTGTTCCCATGGATTTT | GTCTCCTCTGCCACAAGGTC |
| Gata4cDNA | TTTGGGTTTTTGTTTTGTTTTG | CCCAGCCTTTTACTTTGCTG |
| Nr5a1cDNA | TCCAGTGTCCACCCTTATCC | GGGCTCCTGGATCACCTAAT |
| Smad3cDNA | AGCCATGTCGTCCATCCT | ACCCGCTCCCTTTACTCCTA |
| Sox9cDNA | ATGAATCTCCTGGACCCCTTCAT | AAAAAGATCAGCTCTGTCACCA |
| Spz1cDNA | GGAGACTGCCATCTGCTCTC | TGCTTCTGGCGACTTCTACC |
| Wt1cDNA | CTGGACTTCCTCCTGTCGCAGCAG | TCAAAGCGCCAGCTGGAGTTTGGT |
| Zfp239cDNA | CCACATGGCTGAGAAACCTT | TCTTCTGAGTGGGCTCATGTT |

**SUPPLEMENTARY REFERENCES**


Babu, J.R., Jeganathan, K.B., Baker, D.J., Wu, X., Kang-Decker, N., and van Deursen, J.M. (2003). Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. J Cell Biol *160*, 341-353.

Cartharius, K., Frech, K., Grote, K., Klocke, B., Haltmeier, M., Klingenhoff, A., Frisch, M., Bayerlein, M., and Werner, T. (2005). MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics *21*, 2933-2942.