**Supplemental Table 1.** Genotyping primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence (5’- 3’)</th>
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</thead>
</table>
| Zfy    | Forward: CTA-TTG-CAT-GGA-CAG-CAG-TCT-TAT-G  
Reverse: ACT-AGA-CAT-GTC-TTA-ACA-TCT-GTC-C |
| Sf1(Nr5a1)-Cre | Forward: GAG-TGA-ACG-AAC-CTG-GTC-GAA-ATC  
Reverse: GCA-TTA-CCG-GTC-GAT-GCA-ACG-AGT-G |
| Gata4  | Forward: CCC-AGT-AAA-GAA-GTC-AGC-ACA-AGG  
Reverse: AGA-CTA-TTG-ATC-CCG-GAG-TGA-ACA |
| Gata6  | Forward: GTG-GTT-GTA-AGG-CGG-TTT-GT  
Reverse: ACG-CGA-GCT-CCA-GAA-AAA-GT |

**Supplemental Table 2.** Quantitative RT-PCR primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence (5’- 3’)</th>
</tr>
</thead>
</table>
| Gapdh  | Forward: GCT-CAC-TGG-CAT-GGC-CTT-CCG-TG  
Reverse: TGG-AAG-AGT-GGG-AGT-TGCTGT-TGA |
| Amh    | Forward: GCA-GTT-GCT-AGT-CCT-ACA-TCT-GGC-T  
Reverse: TGG-AGG-CTC-TTG-GAA-CTT-CAG-CAA |
| Cyp11a1| Forward: AAG-TAT-GGC-CCC-ATT-TAC-AGG  
Reverse: TGG-GGT-CCA-CGA-TGT-AAA-CT |
| Cyp11b1| Forward: GCT-TCA-CCA-TGT-GCT-GAA-ATC-C  
Reverse: AGA-AGA-GAG-GGC-AAT-GTG-TCA |
| Cyp11b2| Forward: GCA-CCA-GGT-GGA-GAG-TAT-GC  
Reverse: CCA-TTC-TGG-CCC-ATT-TAG-C |
| Cyp21a1| Forward: CGC-TTG-GGG-ATG-CAA-GAT-GTG-G  
Reverse: AGC-ATC-AGG-GCT-GAG-CGA-GAG-A |
| Dhh    | Forward: ATC-CAC-GTA-TCG-GTC-AAA-GC  
Reverse: GTA-GTT-CCC-TCA-GCC-CCT-TC |
| Dmrt1  | Forward: TGG-GTT-CTG-GAA-GCA-AGA-AG  
Reverse: CTG-TCT-TCT-CAG-GGC-CAC-CT |
| Foxl2  | Forward: GCA-AGG-GAG-GCG-GGA-CAA-CAC  
Reverse: GAA-CGG-GAA-CTT-GGC-TAT-GAT-GT |
| Hsd3b1 | Forward: CTC-GTC-AAC-TGG-GAG-GAA-GC  
Reverse: TTT-CCA-TCA-CTG-GCA-CTT-TG |
| Hsd3b6 | Forward: TCC-CCA-TTC-AGA-GCA-TGT-ATA-GC  
Reverse: TTT-TTT-TGA-GGT-ATT-GAC-AAG-TAT-TTA-TTG |
| Hsd17b3| Forward: ATG-GAG-TCA-AGG-AGG-AAA-GGC  
Reverse: GGC-TGT-AAA-GAG-GCC-AGG-G |
| Gata1  | Forward: TGT-CCT-CAC-CAT-CAG-ATT-CCA  
Reverse: TCC-CTC-CAT-ACT-GTT-GAG-CAG |
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
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<tbody>
<tr>
<td>Gata4</td>
<td>AAA-CGG-AAG-CCC-AAG-AAC-CTG-AAT</td>
<td>GAG-CTG-GCC-TGC-GAT-GTC-TGA-GTG</td>
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<tr>
<td>Gata6</td>
<td>AGT-TTT-CCG-GCA-GAG-CAG-TA</td>
<td>AGT-CAA-GGC-CAT-CCA-CTG-TC</td>
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<tr>
<td>Insl3</td>
<td>CAT-GCG-CGC-GCC-GCT-GCT-AC</td>
<td>TCA-GTG-GGG-ACA-CAG-ACC-C</td>
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<tr>
<td>Lhr</td>
<td>GAG-ACG-CTT-TAT-TCT-GCC-ATC-T</td>
<td>CAG-GGA-TTG-AAA-GCA-TCT-GG</td>
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<tr>
<td>Mc2r</td>
<td>TGG-AAA-AGT-TCT-CAG-CAC-CAC</td>
<td>TCT-TTG-TGT-GGA-AGG-ATC-TGG</td>
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<tr>
<td>Mvh</td>
<td>CCA-AGA-TCA-GGG-GAC-ACA-GC</td>
<td>CTT-TGG-CAA-GTG-TCA-CCA-TTG-C</td>
</tr>
<tr>
<td>Sf1(Nr5a1)</td>
<td>CTC-CCT-CTG-GTC-CTC-TTC-CT</td>
<td>TCG-TGG-TAG-TCG-TCG-TAG-TCG-TA</td>
</tr>
<tr>
<td>Sox9</td>
<td>GAG-CCG-GAT-CTG-AAG-AGG-GA</td>
<td>GCT-TGA-CGT-GTG-GCT-TGT-TC</td>
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<tr>
<td>Star</td>
<td>GCA-GCA-GGC-AAC-CTG-GTG</td>
<td>TGA-TTG-TCT-TCG-CA-GCA-GCC</td>
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</tbody>
</table>
Supplemental Figure 1. Assessment of cell proliferation using BrdU in conditional double mutant embryonic testes. Testicular sections from controls (A-E) and SflCre; Gata4^{flox/flox} Gata6^{flox/flox} mice (F-J) at embryonic days (E)15.5 (A-C; E-G) and 17.5 (D, E, I, J) were stained for bromodeoxyuridine (BrdU; green) (A-J); mouse vasa homologue (MVH; red) (B, G, E, J) and Wilms Tumor 1 (WT1; red) (C and H). Nuclei are stained with DAPI (blue). (K) Changes in somatic cell proliferation in SflCre; Gata4^{flox/flox} Gata6^{flox/flox} testes at E17.5. The results are shown as the percentage of BrdU-positive cells relative to the number of DAPI-positive cells from three different males (n = 3) of each genotype.

Supplemental Figure 2. TUNEL assay in embryonic testes. Sections of control (A, D) and SflCre; Gata4^{flox/flox} Gata6^{flox/flox} (B, C, E, F) testes at embryonic days (E) 15.5 (A-C) and 17.5 (D-F). Cell death was assessed by TUNEL using TMR Red conjugated dUTP to identify apoptotic nuclei (yellow) and MVH (green) to identify germ cells. Nuclei were counterstained with DAPI (blue). Panels C and F are higher magnifications of B and E, respectively. Arrows in panels B and E point to TUNEL-positive cells localized close to the coelomic epithelium. Arrowheads in panels C and F show TUNEL- and MVH-positive cells. Scale bars are 100 μm (A, B, D, E) and 50 μm (C, F).

Supplemental Figure 3. Normal testis development in transgene-rescued Gata1 null males (Gata1^{+/Tr}, (35)). Sectioned adult testes from the control (A, B, E-G) or Gata1^{+/Tr} (C, D, H-J) males were stained with antibodies against GATA1 (A, C), H2AX (B, D), GATA4 (E, H), GATA6 (F, I) and AMH (G, J). All proteins (with notable exception of GATA1) are expressed normally in the double mutants. Scale bars represent 100μm (A-D); 50μm (E-J).
Supplemental Figure 4. Whole-mount in-situ hybridization (WISH) comparing Cyp11a1 (A and D), Cyp17a1 (B and E) and Hsd17b3 (C and F) in controls (A-C) and Sf1Cre; Gata4flox/flox Gata6flox/flox (D-E) male gonads at embryonic day (E) 15.5. The dark purple staining represents specific binding of the RNA antisense probes in the gonad. In all panels, the gonad (g) and mesonephros (m) are as indicated in panel A.

Supplemental Figure 5. Ventral view of testicular position in control (A) and Sf1Cre; Gata4flox/flox Gata6flox/flox (B) males at postnatal day (PND) 9. In panels A and B, arrows point to testes. K, kidney; B, bladder. (C) Gross appearance of the seminal vesicles from control (asterisks) and Sf1Cre; Gata4flox/flox Gata6flox/flox (arrowheads) males at PND 90. (D) Seminal vesicle weights in 90 days-old control and Sf1Cre; Gata4flox/flox Gata6flox/flox males. The data were analyzed using Student’s t-test, with significance considered at **P < 0.01. (E) Submaxillary gland weights in 90 days-old control males (black bar), control females (light grey bar) and Sf1Cre; Gata4flox/flox Gata6flox/flox males (dark grey bar). The data were analyzed using ANOVA (one-way) followed by the Sidak's multiple comparisons test. Bars with different superscripts differ significantly (**P < 0.001). The results are graphed as the means ± s.e.m. from n = 3 of each genotype.

Supplemental Figure 6. Testicular function is not altered in Sf1Cre; Gata6flox/flox animals. Sections from controls (A-C) and Sf1Cre; Gata6flox/flox testis (D-O) at postnatal day (PND) 180 were stained for GATA4 (green) (A, D) and GATA6 (red) (B, E); sex determining region Y-box 9 (SOX9; red) (G) and 3β-hydroxysteroid dehydrogenase (3βHSD; green) (H); Wilms Tumor 1
(WT1; red) (J) and anti-Müllerian hormone (AMH; green) (K); mouse vasa homolog (MVH; red) (M) and GATA1 (N). Panels C, F, I, L and O are merged images. Nuclei are stained with DAPI (blue; inset in F; I, L and O). Insets in panels F and O are scaled high-magnification (400x) images. Notice that Sertoli cells lost GATA6 staining, but retained GATA4 (inset in F, arrows, compare to C). Scale bars: 100µm.