<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Company</th>
<th>Code</th>
<th>Dilution</th>
<th>Working Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptavidin Alexa Fluor® 647 conjugate</td>
<td>Invitrogen</td>
<td>S21374</td>
<td>1:333</td>
<td>3 μg/mL</td>
</tr>
<tr>
<td>Streptavidin Alexa Fluor® 546 conjugate</td>
<td>Invitrogen</td>
<td>S11225</td>
<td>1:333</td>
<td>3 μg/mL</td>
</tr>
<tr>
<td>Streptavidin Cy3 conjugate</td>
<td>Jackson Immunoresearch</td>
<td>016-160-084</td>
<td>1:500</td>
<td>1 μg/mL</td>
</tr>
</tbody>
</table>
Table S2. Proportion of ID4+ cells that co-stain for other markers.

<table>
<thead>
<tr>
<th>Marker</th>
<th>% (mean±S.D.)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLZF</td>
<td>96.0±1.8</td>
<td>94.1-97.6</td>
</tr>
<tr>
<td>UTF1</td>
<td>37.1±15.1</td>
<td>24.8-54.0</td>
</tr>
<tr>
<td>KIT</td>
<td>13.3±4.1</td>
<td>8.6-16.3</td>
</tr>
<tr>
<td>Ki67</td>
<td>1.6±1.1</td>
<td>0.6-2.8</td>
</tr>
</tbody>
</table>
Figure S1

(-) control for mouse primary antibodies

A  normal mouse IgG

(-) control for rabbit primary antibodies

B  normal rabbit IgG

C  DNA

D  merge

(-) control for goat primary antibodies with BT amplification

E  normal goat IgG

F  normal rabbit IgG

G  DNA

H  merge

(-) control for mouse primary antibodies with BT amplification

I  normal mouse IgG

J  normal rabbit IgG

K  DNA

L  merge

Figure S1. Negative controls for standard and highly amplified IF assays.
Figure S2. Example of image-based cell quantitation procedure for ID4 IHC staining.
Figure S3

Figure S3. Representative UTF1 IHC in adult testis for young and older donors.
Figure S4

Figure S4. Co-localization of anti-ID4 and anti-UTF1 signals in the human seminiferous epithelium (additional field).
Figure S5. Representative GPR125 IHC in adult testis for young and older donors.
Figure S6. Partial overlap of anti-ID4 and anti-GPR125 signal in the human seminiferous epithelium.
Figure S7. Co-localization of anti-ID4 and either anti-KIT or anti-WT1 immunoreactive cells in the human seminiferous epithelium.