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identified in whole mount preparations of recipient mouse seminiferous tubules using the rabbit anti-primate antibody. Colonies in each recipient testis were counted and normalized to  $10^5$  viable cells transplanted per testis. (**A and B**) For THY1 and EPCAM, no significant difference was found between the unsorted cells and the sorted fractions (P>0.05). (**C**) ITGA6 positive fraction was enriched roughly 3 fold compared to unsorted cells (P<0.05). Bar graphs are presented as mean  $\pm$  SEM. Scale bar =  $100 \mu m$ .

Supplementary figure 1. Isotype controls for (A) ZBTB16 and SALL4, (B) UTF1 and SALL4, (C) SALL4 and KIT, (D) UTF1 and KIT, (E) UTF1 and UCHL1, (F) KIT and UCHL1, (G) ENO2 and UCHL1, (H) ENO2 and SALL4. Scale bars = 100 μm.

Supplementary figure 2. Summary of marker expression in adult human testis. Colored bars indicate the overlap of markers based on data from Figure 1. Shaded area indicates range in data. UTF1 seems to be the most restricted marker of human spermatogonia, followed by ZBTB16 and SALL4. There is also almost no overlap between these markers and differentiation marker KIT. UCHL1 and ENO2 are more widely expressed on the basement membrane of the seminiferous tubule and have slightly more overlap with KIT.

Supplementary figure 3. ITGA6, and EPCAM expression in adult human testis sections. Immunofluorescence staining for ITGA6 (A and C) and EPCAM (D and F) in adult human testis. DAPI staining (blue) (B and E) identifies all the nuclei. Scale bars =  $50 \mu m$ .

**Supplementary figure 4.** Isotype controls for **(A)** THY1 and **(B)** ITGA1 FACS sorting.

Negative gates were defined by analysis of human testis cells stained using **(A)** APC-conjugated and **(B)** PE-conjugated isotype control antibodies.

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Supplementary figure 5. Rabbit anti-primate antibody and ENO2 co-staining of recipient mouse testes xenotransplanted with human testis cells. Immunofluorescence co-staining for the primate antibody (A and C) and ENO2 (B and C) in human to nude mouse xenotransplants testis. DAPI staining (blue) identifies all the nuclei. Scale bars =  $50 \mu m$ .

## Supplementary Table 1. Germ cell markers in rodents, non-human primates and humans.

	Rodents		Nonh	Nonhuman primates			Humans				
	RT- PCR	IHC ICC	TR	RT- PCR	IHC ICC	TR	RT- PCR	IHC ICC	TR	Comments	References
Germ cell*											
ID4	X	IHC-XS IHC-WM	X					\(\lambda\)		$A_{s}$	(18)
EXOSC10							X	IHC-XS		$A_d$	(57)
OCT2								IHC-XS		$A_d$	(59)
FGFR3							X	IHC-XS		$A_d, A_p$	(58)
EGR3		IHC-WM								$A_s, A_{pr}$	(131)
NANOS2	X	IHC-XS IHC-WM	X				7	IHC-XS		$A_s, A_{pr}, A_d, A_p, B-RS$	(30-32, 132, 133)
GFRa1		IHC-XS IHC-WM	X		IHC-XS			IHC-XS		$A_{s}-A_{al4}, \\ A_{d}, A_{p}, B_{1}, B_{2}$	(49, 60-62, 75, 113, 116, 134)
UTF1	X	IHC-XS					X	IHC-XS		$egin{aligned} A_s - A_{al4}, \ A_d,  A_p,  BM \end{aligned}$	(24, 55)
ZBTB16		IHC-XS IHC-WM	X		IHC-XS		X	IHC-XS		$\begin{array}{c} A_s\text{-Aal,} \\ Ad,  A_p,  B_1,  B_2, \\ BM \end{array}$	(13, 14, 22, 60, 61, 75, 102, 103, 135)
SALL4		IHC-XS IHC-WM			IHC-XS	· ·	X	IHC-XS		A <sub>s</sub> -Aal, A <sub>d</sub> , A <sub>p</sub> , B	(56, 102, 103, 135)
POU5F1 (OCT4)		IHC-XS IHC-WM	X		IHC-XS					A <sub>s</sub> -Aal, BM	(136-138)
POU3F1		IHC-XS	X							Spermatogonia on the BM	(37)
RET		IHC-XS								A <sub>s</sub> -Aal,	(9)
BCLB6		IHC-XS	Х							Rare cells on the BM. RS in stage 7	(128, 139)
LHX1		IHC-XS	X	<b>y</b>						Rare cells on the BM. RS in stage 7	(128)
ETV5		IHC-XS	X							On the BM	(128)

NANOS3	X	IHC-XS IHC-WM								A <sub>s</sub> -A1	(30-33)
SOX3		IHC-XS								A <sub>s</sub> -Aal	(140)
GPR125		IHC-XS IHC-WM	X		IHC-XS		X	IHC-XS ICC		A <sub>s</sub> -Aal, rare cells on the BM	(20-22, 60, 135)
LIN28 (TEX17)		IHC-XS IHC-WM			IHC-XS			IHC-XS	R	A <sub>s</sub> -Aal, rare cells on the BM	(19, 63)
UCHL1 (UCHL1)		IHC-XS			IHC-XS		X	IHC-XS	<del>_</del>	Spermatogonia, cells on the BM	(22, 60, 135, 141)
NGN3		IHC-XS IHC-WM	X		IHC-XS				)	$A_s$ -Aal, $A_p$ , $B_{1-4}$ , PS	(28, 29, 75, 140)
SOHLH1	X	IHC-XS IHC-WM						45		GFRα1 <sup>-</sup> spermatogonia	(34, 142)
SOHLH2	X	IHC-XS IHC-WM					_ <			GFRα1 <sup>-</sup> spermatogonia	(34-36, 142)
CBL							X	IHC-XS		BM	(54)
DSG2							$\mathbf{X}$	IHC-XS		BM	(54)
SAGE1								IHC-XS		В	(59)
TRA-1-81					IHC-XS		7			Rare cells on the BM	(135, 143)
MAGEA4					IHC-XS			IHC-XS		BM, some spc	(60, 62, 135, 136, 144)
SNAP91						()	X	IHC-XS		BM, some spc	(54)
RBM	X	IHC-XS				<b>Y</b>		IHC-XS		$A_s$ -A4 $A_d$ , $A_p$ , B-RS	(145-149)
RAR□	X	IHC-XS IHC-WM								A <sub>al</sub> -A1	(150)
KIT	X	IHC-XS IHC-WM	X	X	IHC-XS		X	IHC-XS		$\begin{array}{c} A_{al}\text{-RS} \\ A_p, B_{1\text{-}4}, PS, \\ BM \end{array}$	(60, 75, 76, 103, 112, 116, 151-155) unpublished data
STRA8	X	IHC-XS IHC-WM	X							A <sub>s</sub> -RS	(156-158)
TAF4b		IHC-XS	X							A <sub>s</sub> -Spc	(159)
DAZL	X	IHC-XS		<b>Y</b>	IHC-XS		X	IHC-XS		A <sub>s</sub> -Spc	(22, 82, 160, 161)
VASA		IHC-XS			IHC-XS		X			A <sub>s</sub> -RS	(22, 116, 135, 162, 163)
mRNA-		IHC-XS	X							On the BM	(125)

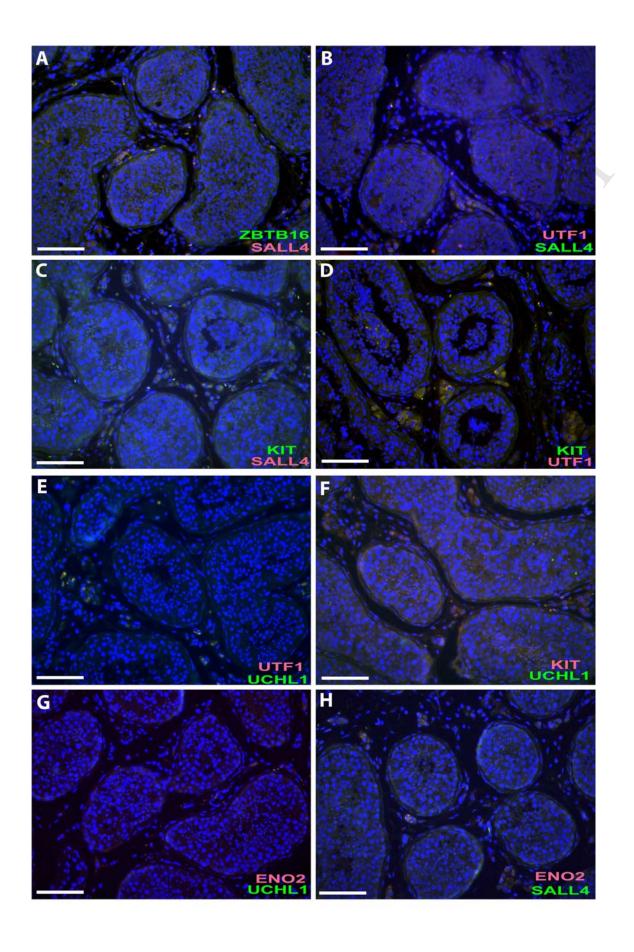
221/222											
mRNA- 146	X									Undifferentiate d spermatogonia	(164)
mRNA-20	X	IHC-XS	X						R	Undifferentiate d spermatogonia	(165)
mRNA- 106a	X	IHC-XS	X						£,	Undifferentiate d spermatogonia	(165)
miRlet7		IHC-XS							)	On the BM	(166)
Cell- surface**								.6			
GFRa1		IHC-XS IHC-WM	X		IHC-XS			IHC-XS		$\begin{array}{c} A_s\text{-}A_{al4}, \\ A_d, A_p, B_1, B_2 \end{array}$	(49, 60-62, 75, 113, 116, 134)
KIT	X	IHC-XS IHC-WM	X	X	IHC-XS		X	IHC-XS		$\begin{array}{c} A_d,A_p,B_1,B_2\\ A_{al}\text{-RS}\\ A_p,B_{1\text{-}4},PS,\\ BM \end{array}$	(60, 75, 76, 103, 112, 116, 151-155)
THY1 (CD90)			X			X	\ \ \	IHC-XS	X		(15, 25, 60, 75, 76, 84, 116) unpublished data
CDH1 (CD324)		IHC-XS IHC-WM	X		<i></i>	\$) <sup>y</sup>				A <sub>s</sub> -A <sub>al</sub>	(17, 167)
CD9		IHC-XS	X			·		IHC-XS	X	BM	(26, 27, 62, 119)
CD29 (β1-integrin)			X	,	2		X	IHC-XS		BM	(16, 23, 151)
CD49f (ITGA6)			X		IHC-XS		X	IHC-XS ICC	X	ВМ	(16, 60, 76, 84, 112, 116, 124, 151) unpublished data
EPCAM		IHC-XS	X				X		X		(22, 25, 26, 81, 168) unpublished data
SSEA4				<b>&gt;</b>	IHC-XS	X		IHC-XS	X		(76, 116, 135, 143, 169)
GPR125		IHC-XS IHC-WM	X		IHC-XS		X	IHC-XS ICC		A <sub>s</sub> -Aal, rare cells on the BM	(20-22, 60, 135)

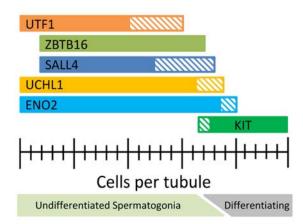
<sup>\*</sup> We attempted to sort the germ cell markers in the order of their expression from undifferentiated to differentiating spermatogonia

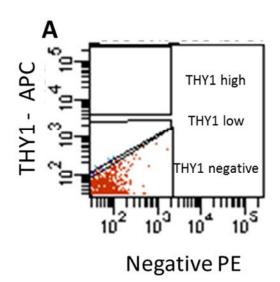
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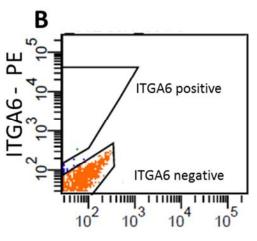
\*\* Examined with flow cytometry or FACS experiments. FGFR3 and TRA-1-81 are also cell surface markers but not included here because the have not been studied in the context of flow cytometry or FACS.

Abbreviations: IHC-XS, immunohistochemistry cross-section; IHC-WM, immunohistochemistry whole-mount; ICC, immunocytochemistry; TR, transplant; As, A single spermatogonia; Apr, A paired sprematogonia; Aal, A aligned spermatogonia; Ad, A dark spermatogonia; Ap, A pale spermatogonia; B, type B spermatogonia; Spc, spermatocytes; RS, round spermatids; PS, pachytene spermatocytes; BM, basement membrane









Negative FITC

