Supplemental Table I Antibodies Used to Target Canine SSC Markers

A: Primary Antibodies

Target Molecule	Host Species	Isotype	Target Species	Vendor	Catalogue #	Optimal Dilution
VASA(DDX4)	Rabbit	IgG	Human	abcam	ab13840	100X
DAZL	Goat	IgG	Human	NOVUS	NB100-2437	50X
UCHL1	Goat	IgG	Human	Santa Cruz	SC-23852	30x
PLZF (A)	Goat	IgG	Human	R and D Syst.	AF2944	40X
PLZF (B)	Mouse	IgG	Human	Santa Cruz	SC-28319	25X
AP180	Rabbit	IgG	Rat	abcam	ab33898	50X
POUF5 (Oct3/4)	Goat	IgG	Human	R and D Syst.	AF1759	30X
RET	Rabbit	IgG	Humam	Santa Cruz	sc-13104	33X
GATA4	Goat	IgG	Mouse	Santa Cruz	sc-1237	50X
Vimentin	Mouse	IgG	Human	Santa Cruz	SC-58901	100X
Alpha Actin	Mouse	IgG	Human	Santa Cruz	SC-69972	100X

B: Secondary Antibodies

Target Molecule	Host Species	Isotype	label	Vendor	Catalogue #	Optimal Dilution
Goat IgG	Donkey	IgG	Alexa 488	Invitrogen	A11055	1000X
Rabbit IgG	Donkey	IgG	Alexa 488	Invitrogen	A21206	1000X
Rat IgG	Donkey	IgG	Alexa 488	Invitrogen	A21208	1000X
Mouse IgG	Donkey	IgG	Alexa 488	Invitrogen	A21202	1000X
Goat IgG	Donkey	IgG	Alexa 594	Invitrogen	A11058	1000X
Rabbit IgG	Donkey	IgG	Alexa 594	Invitrogen	A21207	1000X
Rat IgG	Donkey	IgG	Alexa 594	Invitrogen	A21209	1000X
Mouse IgG	Donkey	IgG	Alexa 594	Invitrogen	A21203	1000X

Supplementary Table II **Culture Conditions Tested for Canine SSC**

Category	Factor[1]	Ref[2]	Conc.	Culture Condition																							
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Base	DME/F12			X	X	X	X																				
Medium	SG	57						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	FBS	9	10%	X				X																			
	CS	9	10%		X																						
Serum	FBS	9	1%			X			X																		
	CS	9	1%				X																				
	TC			X	X	X	X	X	X	X																	
Substratum	Laminin	73	40 ug/ml								X				X	X	X				X	X	X	X	X	X	X
	Gelatin	57	0.10%									X						X	X	X							
Feeder	MEFs	9	2.5E4/cm^2										X			X			X								
Cells	CTFs	9	2.5E4/cm^2											X			X			X							
	GDNF	78	20 ng/ml												X	X	X	X	X	X	X	X	X	X	X	X	X
	bFGF	11	10 ng/ml												X	X	X	X	X	X	X	X	X	X	X	X	X
	EGF	11	10 ng/ml												X	X	X	X	X	X	X	X	X	X	X	X	X
	GFRa1s	1	100 ng/ml												X	X	X	X	X	X	X	X	X	X	X	X	X
	LIF	11	10 ng/ml												X	X	X	X	X	X	X	X	X	X	X	X	X
Growth	BDNF	79	10 ng/ml																		X						X
Factor	FGF9	80	10 ng/ml																			X					X
	NT3	79	10 ng/ml																				X				X
	NT4	79	10 ng/ml																					X			X
	Pleiotrophin	81	10 ng/ml																						X		X
	Wnt3a	82	10 ng/ml																							X	X
Presence of VASA+ cells after 30 days				-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+

1 Reference that indicates factor facilitates survival and/or self renewal of SSC.

2 Abbreviations: (FBS) Fetal bovine Serum. (CS) Adult canine serum. (TC) Tissue culture surface. (MEFs) Mouse embryonic fibroblasts. (CTFs) Canine testis fibroblasts. (GDNF) Glial cell derived neurotrophic factor. (bFGF) Fibroblast growth factor beta. (EGF) Epithelial growth factor. (GFRa1) GDNF receptor alpha 1. (LIF) Leukemia inhibitory factor. (BDNF) Brain derived neurotrophic factor. (FGF9) Fibroblast growth factor 9. (NT3) Neurotrophin 3. (NT4) Neurotrophin 4. (Wnt3a), Wingless-related MMTV integration site 3A protein.

Supplementary Table III RTPCR Primers used for analysis of gene expression

Gene	Accession #	Forward Primer	Reverse Primer	Product Size (bp)
GAB2	XM_542285	CCCAACCTTGTTCACCTTTGAACC	TCCATATTGTCCACCAGGAGGTCC	430
TERT	NM_009354	GGAGCCAGAACATTCCACAGAGAC	CGGCGTCCCTCAACAAGCTTGTC	461
ACTB	NM_001195845	GATGACGATATCGCTGCGCTTGTG	CATCACGATGCCAGTGGTGCGG	455
GAPDH	NM_001003142	CTTCCAGGAGCGAGATCCCGCC	TGTAGCCCAGGATGCCTTTGAGG	601
CYPA	AF243140	CCGTGTTCTTTGACATCGCCGTG	GTTCAGATAAAACAGGAGTTAAGATTC	504
POUF5	XM_538830	TCGTGAAGCCGGACAAGGAGAAG	AGGAACATGTTCTCCAGGTTGCCT	378
KLF4	XM_538779	AGTCTGACATGGCTGTCAGCGAC	ATCGGGTAGCTGAAGCTGCAGGT	439
ITGb1	XM_535143	GATGCCTTACATTAGTACGACGCC	TGCAGATAATGTTCCTACTGCTGAC	510
PLZF	XM_845250	CTGTGTCCATGGACTTCAGCACC	TGCACTCGCTGCAGATGTAGCTG	531
RET	XM_543915	CAAAGGGATCACCAGAAACTTCTCC	AGGGAATTCCCACTTAGGATCCTC	513
ITGA6	XM_535964	GCTCTATTTGGTGAAAGTCGAGTCC	CACAGTCACACGAATCTGAGTGCC	409
GPR125	XM_545977	AATAGATGGCGTGAATGTGGATACC	GGGTTATTCCTCCCACGAAGACC	434
NCAD	XM_537293	TCACTGCTATTGATGCTGATGATCC	GCTGATCCTGTACGCAGCATTCC	404
FGF9	XM_844845	GTTGGGAACTATTTCGGTGTGCAG	ACATAGTATCGCCTTCCAGTGTCC	477
VASA	XM_544339	GTGCAGGTAATGGTGACACTTACC	AGCACAAGCCATCAAATCTCGTCC	425
GFRA1	XM_846994	GGGAAATGATCTGCTCGAAGATTCC	AAATTCAGGCAGTTGGGCTTCTCC	405
LIFR.	NM_001005760	ACAGTTCATCACTGGAGTTGGACC	ATGCGGTTGGTCTTCCTGGATTCC	463
NANOS2	XM_541547	CAGCCTTCGACATGTGGAAGGAC	CTCCTGATCCTCAGCGCTTGACC	406
NANOS3	XM_542028	TACACTGCCCAGCTATGGGAACC	GTGGTGTAGCTGTAGACAGAGGTG	382
NRG1	XM_853176	GAGCATATGTGTCTTCAGAGTCTCC	TGAGGCCCGTTTGCTATGTTCACC	448
PIWI 1(B)	XM_534638	TATTATAGTAAGGCTGAGCACTAACC	TTGAAGGATAGAGGTGGTGAAGCC	448
PIWI2	XM_543251	TATCAGCCTGACTTGTCGAGATCC	CTTAATGTTGATATCTGCGCCATCAG	406
FGFR3	XM_545926	GGCCATCGGTATTGACAAGGACC	TAATAGTCGAGGTTGTGCACGTCC	454
FZD3	XM_543219	CAGCTTTAGCAATGGAGCCATTCC	GGAGGTGAACAATCACGCACGTG	392
DAZL	XM_534251	AGCTGAAACTGGGACCTGCAATCAG	ACCACAGTCTGTATGCTTCTGTCC	447
AP180	NM_001194957	TGACCTTGACTCATCTCTTGCCAG	GATGTTAAGATCCGCTAATGGGTCC	444
UCHL1	XM_536245	TAAGGTGTACTTCATGAAGCAGACC	GCATTAGGCTGCCTTGCAGAGTG	444
PTN	XM_532732	GCCAGAAGACAGTCACCATCTCC	ACTTACCTCAATGTTCTAGCATGTAC	378
RTPRZ1	XM_539545	CAGCAAATTCAACAGGAAGGGACC	TGACCATCAGGAAGCATGACTACC	418

SupplementaryTable IV SSC Transplant Information

Recipient			Donor					Harvest
Animal #	**Breed**	**Testis**	**SSC Prep**	**Breed**	**Age**	**# Cells Injected**	**Transduced Reporters**	**Sperm / epididymous**
795933	Hound	R	SSC15	Sheba Inu	4 m	8.0E+05	none	
		L	SSC17	Choc. Lab	2 m	8.0E+05	none	
795941	Hound	R	SSC24	Rat Terrier	7 m	5.5E+05	EF1α-EGFP	1.10E+08
		L	SSC25	Lab mix	10 w	2.0E+06	EF1α-EGFP	1.80E+08
795917	Hound	R	SSC26	Lab/Poodle	6 m	2.0E+05	Stra8-mCherry	4.60E+08
		L	SSC21	Pit Bull	3 m	7.2E+05	EF1α-EGFP	2.90E+08

Supplementary Table V Primers Used for Detection of Donor Sperm

A: Reporter Vector Targets for Distinguishing Transgenic Donor Cells

Region of Vector	Source Sequence	Forward Primer	Reverse Primer	Size (bp)
5' LTR and adjacent	PL-SIN-EF1α-EGFP *	CTCAGACCCTTTTAGTCAGTGTGG	CTGAAGGGATGGTTGTAGCTGTCC	397
3' LTR and adjacent	PL-SIN-EF1α-EGFP *	TTTAAGACCAATGACTTACAAGGCAG	CCACACTGACTAAAAGGGTCTGAG	286
GFP coding	PL-SIN-EF1α-EGFP *	AAACGGCCACAAGTTCAGCGTGTC	CCTTGAAGAAGATGGTGCGCTCC	239
canine cMyc gene	Acc # X95367	AGAATTCATGACCGATTCCAGTTCC	CTTAAGAGATGCCATGTGTCCACC	473

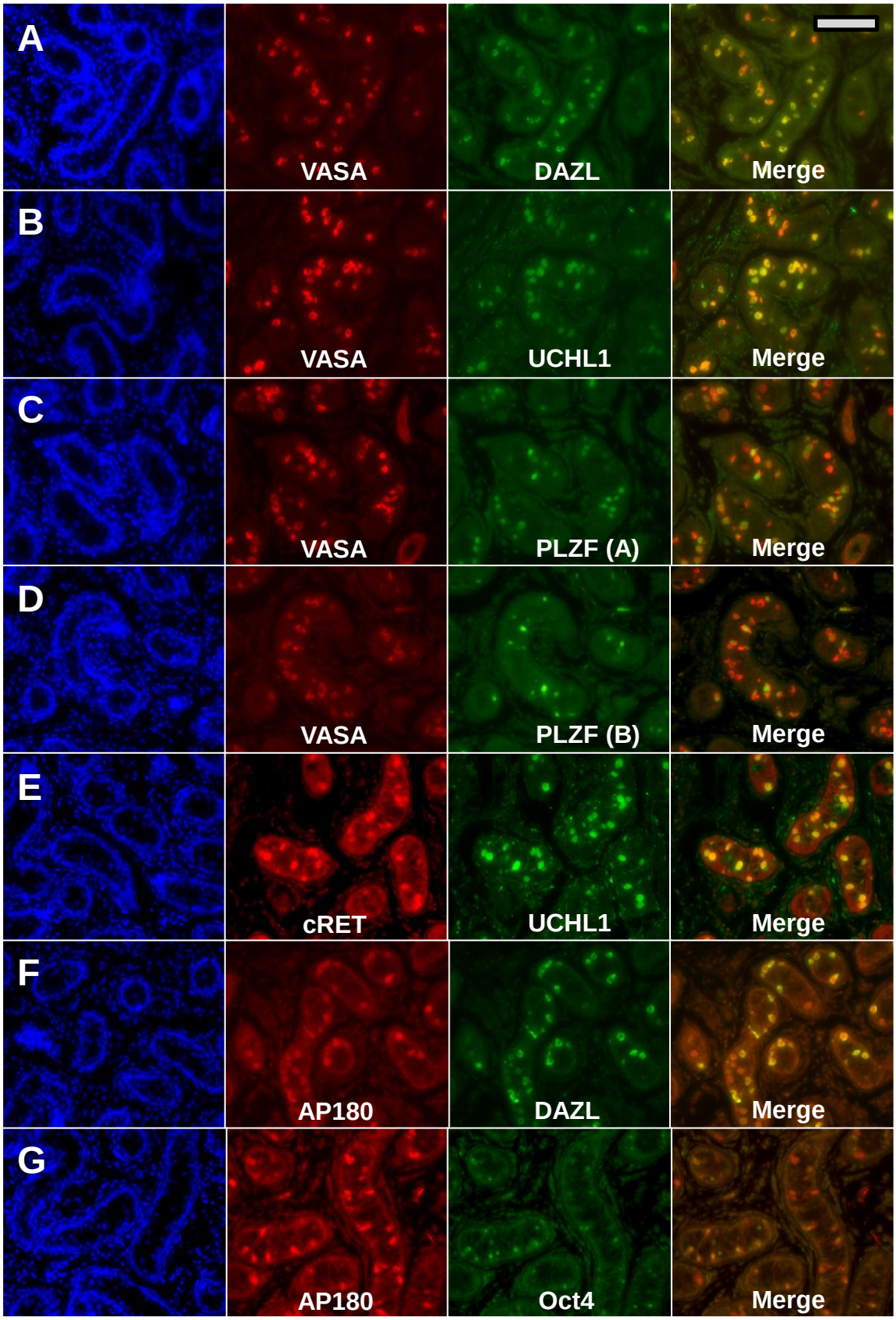
B: Microsatellite Targets for VNTR Analysis of Donor / Recipient Chimerism of Sperm

Locus	Chromosome Position	Forward Primer	Reverse Primer
FH 2001	23:50,961,275	TCCTCCTCTTCTTTCCATTGG	5FAM-TGAACAGAGTTAAGGATAGACACG
FH2199	31:7,285,015**	GCTGAGCACTGGGTATTGTATG	6FAM-TGTTACAAATTAATGTGAAATGGC
FH2611	36:9,233,642	GAAGCCTATGAGCCAGATCA	6FAM-TGTTAGATGATGCCTTCCTTCT

(*) Lenti vector reported by Hotta et al. (2009) and available through Addgene (https://www.addgene.org/) as plasmid # 21320

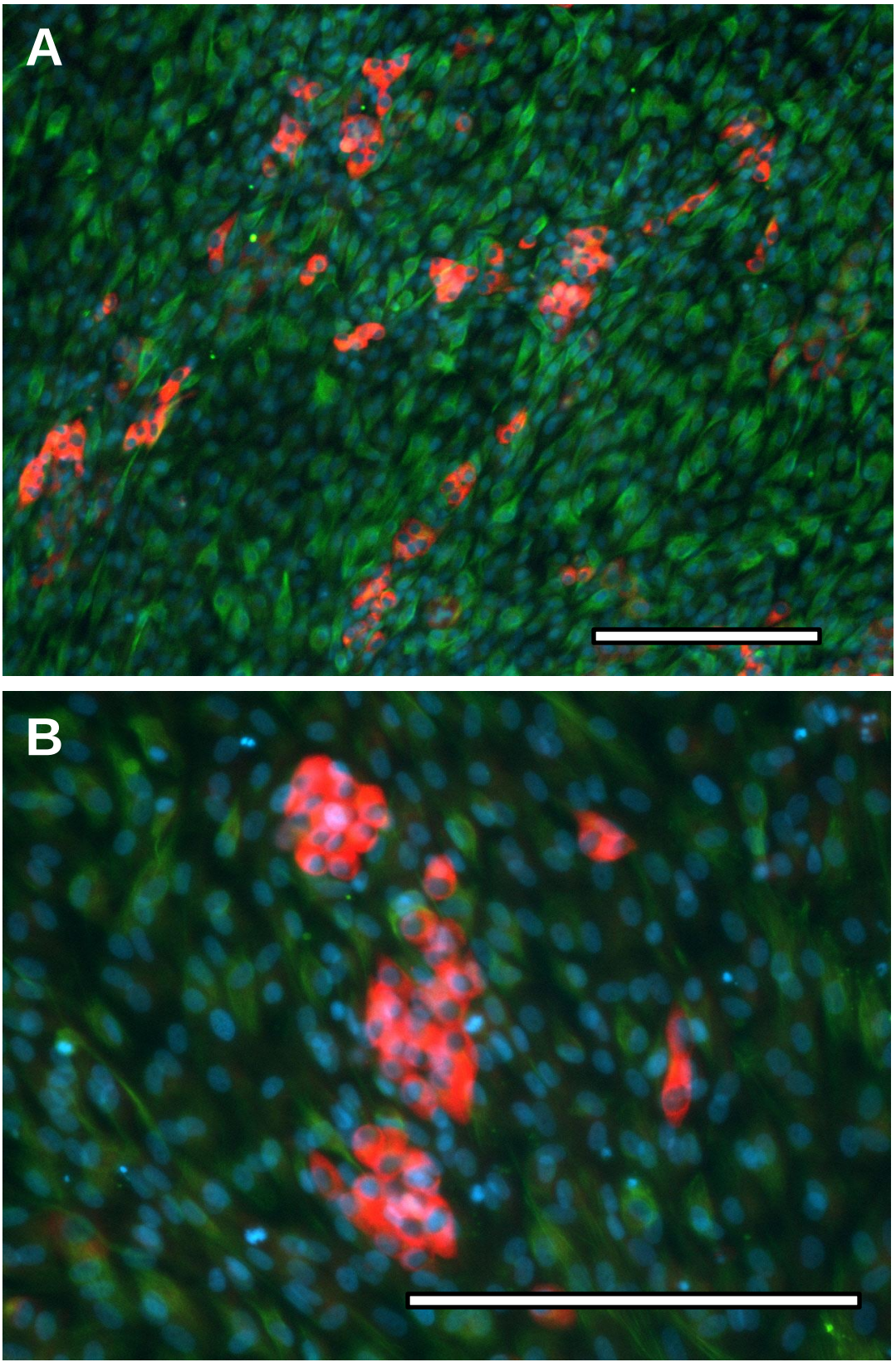
(**) Exact position uncertain due to uncertainties in canine genome at this locus

Supplemental Figure 1: Co-expression of SSC antigens in testis.



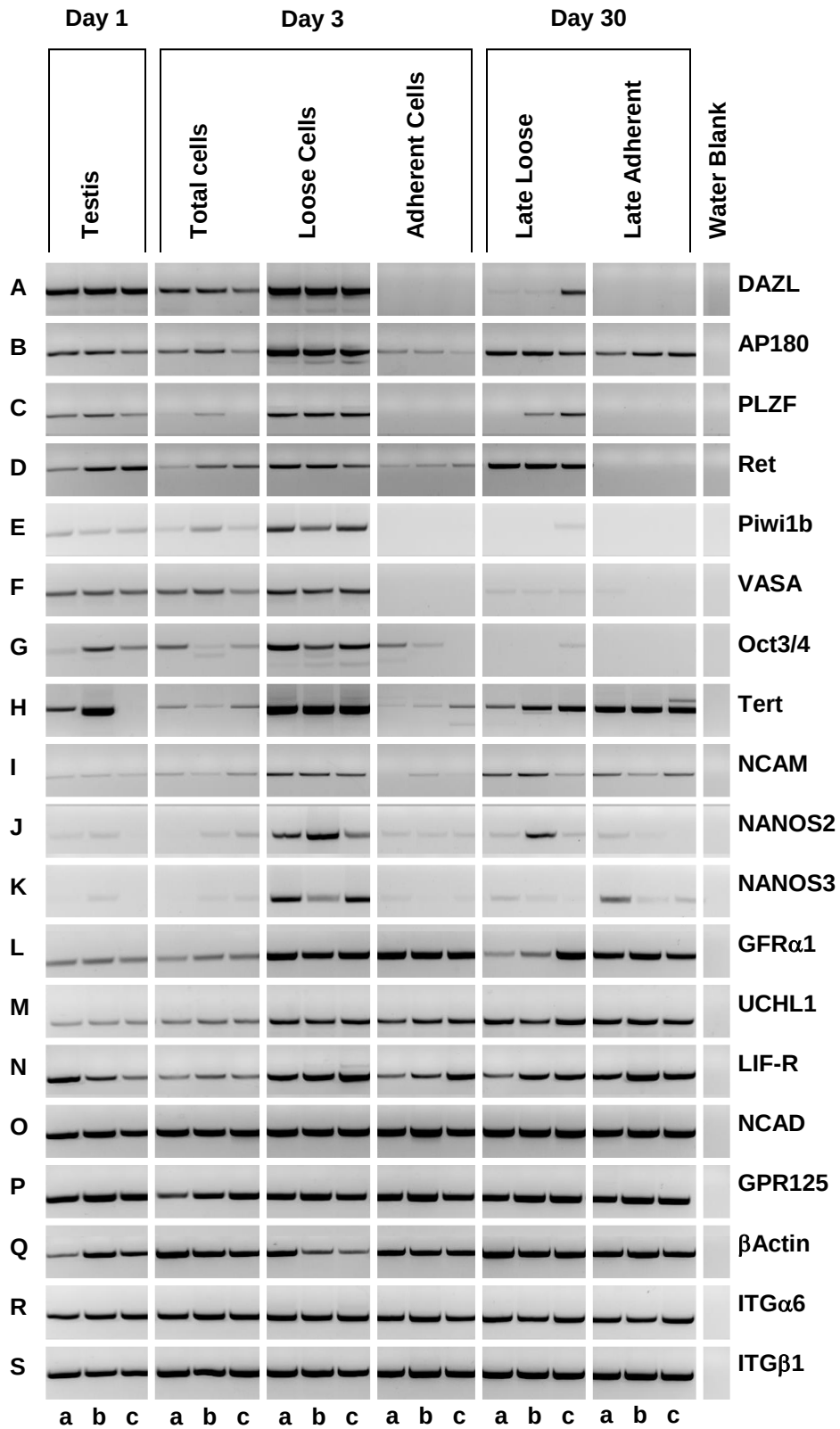
Supplemental Figure 1. Co-localization of SPG Markers in Canine Testis. Paraffin sections of pre-pubertal canine testis were probed by immunofluorescence for pairs of the SPG markers identified in Fig. 1. Appropriate secondary antibodies labeled with Alexa-594 (red) and Alexa-488 (green) were used to distinguish the targets. Sections were counter-stained with Hoechst 33342 (blue) to label nuclei. In each row, the first 3 images show localization of individual probes, and the final image (right) merges the two antibody probe images. Individual targeted antigens are indicated for each image. Bar = 100 uM.

Supplemental Figure 2: Double Immunofluorescence of 30 Day SSC Culture



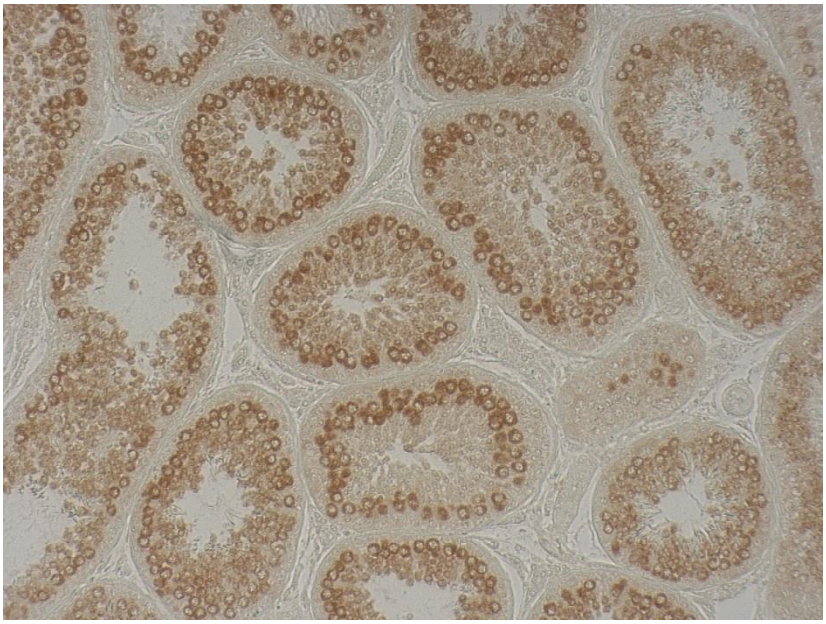
Supplemental Figure 2. Double-Immunofluorescence to Distinguish SPG from Somatic Cells in Culture . After 30 days culture of enriched SPG, the cells were fixed in formalin and probed for VASA as a germ cell marker (red) and both vimentin and alpha actin as somatic cell markers (green). Appropriate secondary antibodies labeled with Alexa-594 (red) and Alexa-488 (green) were used to distinguish the targets. Cells were counter-stained with Hoechst 33342 (blue) to label nuclei. VASA-positive germ cells are clearly distinguishable from the lawn of rapidly expanding somatic cells in the culture. Bar = 100 uM.

Supplemental Figure 3: The SSC-specific expression profile is lost after 30 days culture of "loose" cells.



Supplemental Figure 3. The SPG -Specific Expression Profile is Lost after 30 Days in Culture). The expression analysis from SPG cultures in Figure 4D was extended to 30 days of culture. Day 1 and Day 3 data from Figure 4D are repeated here for comparison. Duplicate plates of freshly isolated SPG (loose cells), and adherent cells were cultured from each of the three testis cell suspensions (a,b, and c) were cultured for an additional 30 days, and analyzed for expression of the designated mRNAs by RTPCR. The RTPCR reactions for 3-day and 30-day samples were done at the same time under identical conditions. It is evident that the enrichment of SPG marker mRNAs seen at day 3 (lines A-K), is mostly lost by day 30.

Supplemental Figure 4: Histology and VASA Expression in Testis 14 Months Post-Transplant.



Supplemental Figure 4. Normal Histology and VASA Expression in Seminiferous tubules 14 Months Post-Transplant. A representative paraffin section of a testis from dog 795941, that was probed for VASA using DAPI immunohistochemistry. Note that all tubules contain VASA-positive cells and show the normal histology of a fully functional spermatogenic seminiferous tissue, indicating full recovery of spermatogenesis. This is representative of all observed sections from all three animals in this study.