

Schmidt et al., SUPPLEMENTAL TABLE S1. Sequences of primers used for qRT-PCR.

Gene <sup>a</sup>	Forward Primer (5'-3')	Reverse Primer (5'-3')
<i>Bcl6b</i>	CATTTTCGGCACAAGAGTCA	TTAGATGGTGGGGACTCAGC
<i>Bhlhe40</i>	GACCGGATTAACGAGTGCAT	TCAATGCTTTCACGTGCTTC
<i>Cd97</i>	GCCAGGCTCTGTTGAGTTTC	AGCCTCGATGAGTTTCTGGA
<i>Egr3</i>	TTTGCCTGTGAGTTCTGTGG	CCCCTTTCTCCGACTTCTTC
<i>Etv5</i>	TGAGAGGGGACCGAGTTATG	CTTCCCTATGGGTGTGCACT
<i>Gbp2</i>	CTCGACTGTGCATCAGGAAA	TGCAGAGTTGCTTCCTCAGA
<i>Hoxc4</i>	TAAACAAACAAGCCCCCTTGC	CCAATGGCCTTAAAAGCAAA
<i>Ldlr</i>	GGATGATGTGGCATGAACAG	GTGAGAAAGCGTCCTTCTCG
<i>Nanog</i>	GGTGAAGACCTGGTTCCAAA	AGGCCGTTGCTAGTCTTCAA
<i>Pou5f1</i>	AGAACCGTGTGAGGTGGAAC	CACCTCGAACCACATCCCCTCT
<i>Rps2</i>	TACCTGACCCCTGACCTCTG	TGGTGTGGGTTTTCAACAAGA
<i>Sox2</i>	ATTACCCGCGAGCAAAATGAC	CTAGTCGGCATCACGGTTTTT
<i>Tcfap2c</i>	CTGAGAACCTAGGGCTGCAC	GGTCCTTTGCGAATGACAGT
<i>Tec</i>	GCCTTCGTTTGAAGACTTGC	TTTCTTCTCTTGCCCTTGGA

<sup>a</sup>*Bcl6b* (previously known as *Bazf*, B-cell CLL/lymphoma 6, member B (zinc finger protein)), *Bhlhe40* (previously known as *Bhlhb2*, basic helix-loop-helix family, member e40), *Cd97* (CD 97 molecule), *Egr3* (early growth response 3), *Etv5* (previously known as *Erm*, ets variant 5), *Gbp2* (guanylate binding protein 2), *Hoxc4* (homeobox C4), *Ldlr* (low density lipoprotein receptor), *Nanog* (nanog homeobox), *Pou5f1* (previously known as *Oct4*, POU class 5 homeobox 1), *Rps2* (ribosomal protein S2), *Sox2* (SRY (sex determining region Y)-box 2), *Tcfap2c* (transcription factor AP-2, gamma), *Tec* (tec protein tyrosine kinase).

Schmidt et al., SUPPLEMENTAL TABLE S2. siRNAs used for functional analysis of gene activity.

Gene <sup>a</sup>	Company	Species <sup>b</sup>	Product no.	Knockdown (%) <sup>c</sup>
<i>Bcl6b</i>	Dharmacon	M, NM_007528	M-043010-00-0005	37.2
<i>Bhlhe40</i>	Ambion	R, NM_053328	AM16708, 195437	48.5
	Dharmacon	R, NM_053328	M-091915-00-0010	54.8
<i>Etv5</i>	Dharmacon	R, XM_221387	M-087219-00-0010	42.9
<i>Hoxc4</i>	Ambion	M, NM_013553	AM16708, 159290	60.6
	Dharmacon	R, XM_347337	M-082152-00-0010	41.8
<i>Tec</i>	Ambion	M, NM_013689	AM16708, 188351	50.8
	Dharmacon	R, XM_341208	M-084511-00-0010	48.2
Neg. Control	Ambion		4611	

<sup>a</sup>*Bcl6b* (previously known as *Bazf*, B-cell CLL/lymphoma 6, member B zinc finger protein), *Bhlhe40* previously known as *Bhlhb2*, basic helix-loop-helix family, member e40), *Etv5* (previously known as *Erm*, ets variant 5), *Hoxc4* (homeobox C4), *Tec* (tec protein tyrosine kinase).

<sup>b</sup>Accession number of the gene utilized to develop the particular siRNA for either mouse (M) or rat (R) genes.

<sup>c</sup>Percent knockdown of the gene of interest by the specific siRNA compared to negative control siRNA in long-term cultures of self-renewing rat SSCs.

Schmidt et al., SUPPLEMENTAL TABLE S3. Numbers of genes present and GDNF-regulated in cultures of rat EPCAM+ clump forming germ cells.

Gene expression	Number
Total expressed by Rat EPCAM+ clump forming germ cells	21,271
Total GDNF-regulated	7,948
Total up-regulated by GDNF	3,741
Total down-regulated by GDNF	4,207
Total up-regulated between control and 18 h GDNF withdrawal 2-fold or greater	113
Total down-regulated between control and 18 h GDNF withdrawal 2-fold or greater	24
Total up-regulated between control and 18 h GDNF withdrawal 2-fold or greater that recovered 2-fold or greater after GDNF replacement	61
Total down-regulated between control and 18 h GDNF withdrawal 2-fold or greater that recovered 2-fold or greater after GDNF replacement	16

Schmidt et al., SUPPLEMENTAL TABLE S4. Functional clustering of GDNF-regulated genes.

Cluster	No. of genes	P value	Fold Enrichment
Genes up-regulated 2-fold between control and 18h GDNF withdrawal <sup>a</sup>			
Extracellular Space	20	0.0001	2.5
Plasma Membrane	19	0.00045	2.3
Ion Binding	17	0.0073	2.0
Cation Binding	15	0.0089	2.1
Metal Binding	12	0.002	2.8
Calcium Ion Binding	10	0.0071	4.0
Genes up-regulated 2-fold between any time point and 18h GDNF withdrawal <sup>a</sup>			
DNA Binding	33	0.00017	2.0
Transcription Regulation	31	0.000023	2.3
Morphogenesis	29	0.000025	2.4
Cell Differentiation	25	0.00021	2.3
Nuclear Protein	25	0.0012	2.0
Metal Binding	23	0.001	2.1
Zinc Interacting	17	0.0025	2.3
Cell Motility	14	0.000574	3.1
Lipid Metabolism	12	0.04	2
Cell Cycle Regulation	11	0.016	2.4
Calcium Ion Binding	10	0.047	2.1

<sup>a</sup>Clusters listed had 10 or more genes per functional group, and a p-value and fold change of  $\leq 0.05$  and  $\geq 2$  for enrichment compared to background.

Schmidt et al., SUPPLEMENTAL TABLE S5. Expression of genes that have been reported to be important for SSC self-renewal, as SSC markers, or for SSC differentiation.

Gene	-18 h <sup>c</sup>	2 h <sup>c</sup>	4 h <sup>c</sup>	8 h <sup>c</sup>	Raw signal <sup>d</sup>
SSC Self-Renewal <sup>a,b</sup>					
<i>Bcl6b</i> <sup>e</sup> [2]	-9.56	2.32	4.03	3.83	1170.39
<i>Etv5</i> <sup>e</sup> [2]	-1.46	-1.09	1.46	1.42	765.94
<i>Fos</i> <sup>e</sup> [5]	-1.17	2.55	1.43	-1.02	1789.85
<i>Lhx1</i> [2]	-1.02	1.01	1.01	1.01	8.84
<i>Zbtb16</i> [6]	-1.58	-1.28	-1.07	1.17	32.43
<i>Stat3</i> [7]	1	1.06	1.1	-1.01	463.81
<i>Piwil2</i> [8]	1.1	1.06	1.02	-1.01	1534.99
<i>Mycn</i> [9]	-1.01	-1.03	1.00	-1.07	862.41
<i>Pou5f1</i> <sup>f</sup> [10]	-1.13		1.27		
<i>Msi2</i> <sup>g</sup> [11]	1.12	-1.01	1.23	-1.12	272.74
<i>Ret</i> <sup>g</sup> [12]	-1.1	-1.39	-1.98	-1.38	2424.4
<i>Gfra1</i> <sup>g</sup> [12]	1.19	1.01	-1.06	1.08	7.81
<i>Bmi1</i> <sup>g</sup> [13]	1.1	-1.16	-1.07	-1.1	1493.95
SSC Markers					
<i>Tacstd1</i> <sup>e</sup> [1]	-1.45	1.04	1.3	1.33	10993.7
<i>Itga6</i> <sup>e</sup> [14]	-1.32	-1.06	-1.11	1	4517.49
<i>Thy1</i> [14]	-1.07	1.01	-1.09	1	5.38
<i>Cd9</i> [15]	-1.29	-1.02	-1.11	-1.03	6783.18
<i>Gpr125</i> <sup>g</sup> [16]	1.04	-1.15	-1.15	-1.09	376.67
SSC differentiation <sup>h</sup>					
<i>Kit</i> <sup>g</sup> [17]	1.71	-1.09	-1.55	-1.53	95.61
<i>Sohlh1</i> [18]	-1.05	1.06	1.03	-1.03	1489.99

<sup>a</sup>Gene symbol (Gene name; Affymetrix probe set identification): *Bcl6b* (previously known as *Bazf*, B-cell CLL/lymphoma 6, member B; 1394375\_x\_at, 1386833\_at, 1386832\_a\_at, 1381804\_at), *Etv5* (previously known as *Erm*, ets variant 5; 1383447\_at), *Fos* (previously known as *c-fos*, FBJ osteosarcoma oncogene; 1375043\_at), *Lhx1* (LIM homeobox 1; 1389982\_at, 1377206\_at), *Zbtb16* (previously known as *Plzf*, zinc finger and BTB containing domain 16; 1381553\_at), *Stat3* (signal transducer and activator of transcription 3; 1370224\_at, 1371781\_at), *Piwil2* (piwi-like 2 (Drosophila); 1384745\_at), *Mycn* (previously known as *n-myc*, v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian); 1376648\_at), *Pou5f1* (previously known as *Oct4*, POU class 5 homeobox 1), *Msi2* (Musashi homolog 2 (Drosophila); 1380541\_at, 1385427\_at, 1388870\_at, 1390813\_at), *Ret* (ret proto-oncogene; 1370989\_at, 1371112\_at, 1388208\_a\_at), *Gfra1* (GDNF family receptor alpha 1; 1367954\_at, 1387007\_at), *Bmi1* (Bmi1 polycomb ring finger oncogene; 1373568\_at), *Epcam* (previously known as *Tacstd1*, epithelial cell adhesion molecule; 1388199\_at), *Itga6* (integrin, alpha 6, 1383240\_at, 1393558\_at, 1371186\_at), *Thy1* (previously known as *CD90*, Thy-1 cell surface antigen; 1369651\_at), *Cd9* (CD9 molecule; 1371499\_at), *Gpr125* (G protein-coupled receptor 125; 1377679\_at, 1385807\_at), *Kit* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; 1369822\_at), *Sohlh1* (spermatogenesis and oogenesis specific basic helix-loop-helix 1; 1385654\_at).

<sup>b</sup>Additional putative SSC self-renewal genes: *Nanos3* (nanos homolog 3; 1397827\_at) was not expressed by the EPCAM+ clump forming germ cells [19]. *Msi1* (Musashi homolog 1 (Drosophila)) [11], *Taf4b* (TAF4B RNA polymerase II, TATA box binding protein (TBP)-associated factor) [20], and *Nanos2* (nanos homolog 2) [21] were not annotated on the array.

<sup>c</sup>Fold change in gene expression after GDNF withdrawal and replacement. -18 h represents the change in expression after an 18 hour GDNF withdrawal compared to before the withdrawal. 2 h, 4 h, and 8 h represent the change in gene expression level after GDNF was replaced for 2, 4, and 8 hours compared to the 18 hour withdrawal.

<sup>d</sup>Average of the raw signals of gene expression on the microarray of 3 replicates of rat EPCAM+ clump forming germ cells prior to GDNF withdrawal.

<sup>e</sup>Genes that were significantly up-regulated by GDNF.

<sup>f</sup>*Pou5f1* was not annotated on the array; however, gene expression was determined using qRT-PCR for time 0, 18 h GDNF withdrawal, and 4 hour GDNF replacement.

<sup>g</sup>Genes that were significantly down-regulated by GDNF.

<sup>h</sup>Additional putative SSC differentiation genes: *Neurog3* (neurogenin 3; 1369853\_at) [22] was not expressed by the EPCAM+ clump forming germ cells. *Stra8* (stimulated by retinoic acid gene 8) [23], *Sox3* (SRY(sex determining region Y)-box containing gene 3) [24], and *Dazl* (deleted in azoospermia-like) [25] were not annotated on the array.

Schmidt et al., SUPPLEMENTAL TABLE S6. Expression of genes in EPCAM+ clump forming germ cells that have been reported to be important for stem cell function in cells other than SSCs.

Gene <sup>a,b</sup>	Stem cell type <sup>c</sup>	-18 h <sup>d</sup>	2 h <sup>d</sup>	4 h <sup>d</sup>	8 h <sup>d</sup>	Raw signal <sup>e</sup>
<i>Pou3f1</i> <sup>f</sup>	NSC, ESC [26]	-1.19	1.42	2.11	1.37	163.58
<i>Egr1</i> <sup>f</sup>	HSC [27]	-1.59	2.71	2.19	1.46	3595.19
<i>Id4</i> <sup>f</sup>	NSC [28]	-1.23	1.44	1.36	1.33	38.31
<i>Tall1</i> <sup>f</sup>	HSC [29]	-1.29	1.07	1.17	1.33	10.12
<i>Klf4</i> <sup>f</sup>	ESC [30]	-1.09	1.53	1.28	-1.03	354.48
<i>Stat3</i>	ESC [31]	1.0	1.06	1.1	-1.01	463.8
<i>Pou5f1</i> <sup>g</sup>	ESC [10]	-1.13		1.27		
<i>Nanog</i> <sup>g</sup>	ESC [32]	-1.34		1.09		
<i>Myc</i>	HSC [33]	-1.19	1.17	1.2	1.26	11.99
<i>Lin28</i>	ESC [34]	-1.22	-1.19	1.17	1.04	13.44
<i>Bmi1</i> <sup>h</sup>	HSC [35]	1.1	-1.16	-1.07	-1.1	1493.95

<sup>a</sup>Gene symbol (Gene name; Affymetrix probe set identification): *Pou3f1* (previously known as *Oct6*, POU class 3 homeobox 1; 1370432\_at), *Egr1* (early growth response 1; 1368321\_at), *Id4* (inhibitor of DNA binding 4; 1375120\_at), *Tall1* (T-cell acute lymphocytic leukemia 1; 1389342\_at), *Klf4* (Kruppel-like factor 4 (gut); 1387260\_at), *Stat3* (signal transducer and activator of transcription 3; 1370224\_at, 1371781\_at), *Pou5f1* (previously known as *Oct4*, POU class 5 homeobox 1), *Nanog* (nanog homeobox), *Myc* (myelocytomatosis oncogene; 1368308\_at), *Lin28* (lin-28 homolog (C. elegans); 1393904\_at), *Bmi1* (Bmi1 polycomb ring finger oncogene; 1373568\_at).

<sup>b</sup>Additional stem cell genes: *Sox2* (SRY (sex determining Y)-box 2, ESC [36]; 1381874\_at, 1375153\_at, 1379942\_at, 1389826\_at), *Dppa3* (previously known as *Stella*, developmental pluripotency-associated 3, ESC [37]), *Foxd3* (forkhead box D3, ESC [38]; 1393646\_at), and *Hoxb4* (homeobox B4, HSC [39]; 1386125\_at) were not expressed by the rat EPCAM+ clump forming germ cells. *Utf1* (undifferentiated embryonic cell transcription factor 1, ESC [40]) was not annotated on the array.

<sup>c</sup>NSC (Neuronal Stem Cell), ESC (Embryonic Stem Cell), HSC (Hematopoietic Stem Cell).

<sup>d</sup>Fold change in gene expression after GDNF withdrawal and replacement. -18 h represents the change in expression after an 18 hour GDNF withdrawal compared to before the withdrawal. 2 h, 4 h, and 8 h represent the change in gene expression level after GDNF was replaced for 2, 4, and 8 hours compared to the 18 hour withdrawal.

<sup>e</sup>Average of the raw signals of gene expression on the microarray of 3 replicates of rat EPCAM+ clump forming germ cells prior to GDNF withdrawal.

<sup>f</sup>Genes that were significantly up-regulated by GDNF.

<sup>g</sup>*Pou5f1* and *Nanog* were not annotated on the array; however, gene expression was determined using qRT-PCR.

<sup>h</sup>Genes that were significantly down-regulated by GDNF.

Schmidt et al., SUPPLEMENTAL TABLE S7. Expression of GDNF-regulated genes identified in mouse SSCs [2] in cultured rat EPCAM+ clump forming germ cells.<sup>a</sup>

Gene <sup>b</sup>	Mouse/Rat-GenBank No.	-18 h <sup>c</sup>	2 h <sup>c</sup>	4 h <sup>c</sup>	8 h <sup>c</sup>
<i>Bcl6b</i>	Rat- XM_001079767	-9.56	2.32	4.03	3.83
	Mouse-NM_007528	-6.23	2.28	2.82	3.89
<i>Egr2</i>	Rat- NM_053633	-1.27	26.12	6.79	1.50
	Mouse-NM_010118	-5.81	4.20	5.25	3.76
<i>Egr3</i>	Rat-NM_017086	-7.21	9.96	9.97	4.50
	Mouse-NM_018781	-3.64	8.26	11.2	3.61
<i>Etv5</i>	Rat- XM_221387	-1.46	-1.09	1.46	1.42
	Mouse-NM_023794	-3.32	2.11	2.82	2.43
<i>Lhx1</i> <sup>d</sup>	Rat-NM_145880	-1.02	1.01	1.01	1.01
	Mouse-NM_008498	-3.94	2.11	2.82	2.43
<i>Tspan8</i> <sup>e</sup>	Rat-NM_133526	NP	NP	NP	NP
	Mouse-NM_146010	-5.92	2.2	2.26	2.83

<sup>a</sup>Data for the fold change of expression of GDNF-regulated genes identified in mouse germ cell clumps was reported previously [2].

<sup>b</sup>*Bcl6b* (previously known as *Bazf*, B-cell CLL/lymphoma6, member B), *Egr2* (early growth response 2), *Egr3* (early growth response 3), *Etv5* (previously known as *Erm*, ets variant 5), *Lhx1* (LIM homeobox 1), *Tspan8* (tetraspanin 8).

<sup>c</sup>Fold change in gene expression after GDNF withdrawal and replacement. -18 h represents the change in expression after an 18 h GDNF withdrawal compared to before the withdrawal. 2 h, 4 h, and 8 h represent the change in gene expression level after GDNF was replaced for 2, 4, and 8 h compared to the 18 h withdrawal.

<sup>d</sup>*Lhx1* expression in rat EPCAM+ clump forming germ cells was not GDNF-regulated.

<sup>e</sup>*Tspan8* was not expressed by rat EPCAM+ clump forming germ cells. NP: Not Present.