Supporting Information

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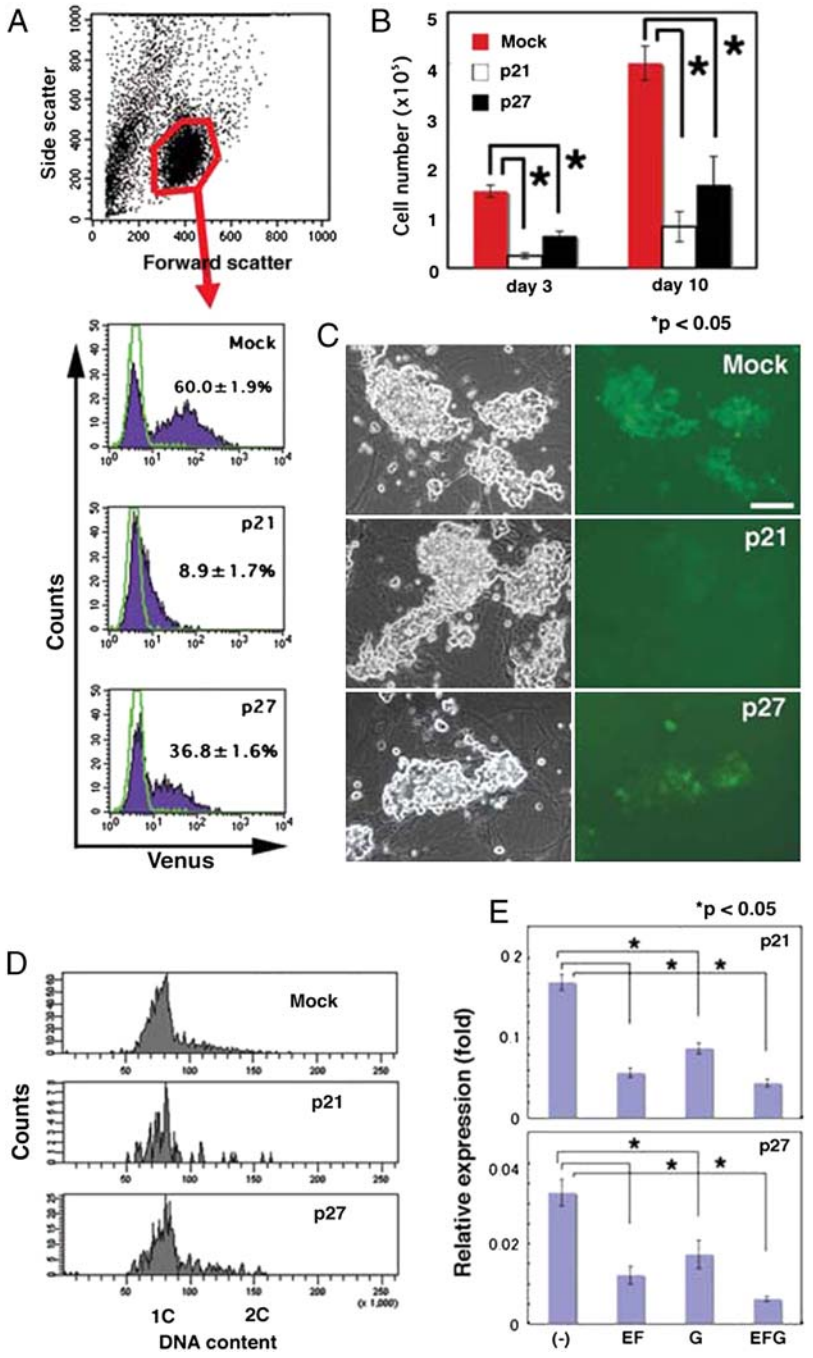

Fig. S1. Functional analysis of p21 and p27 genes. (*A*) Flow cytometric analysis of GS cells at 3 d postinfection by lentiviruses expressing Venus and p21 or p27. Controls are indicated by green lines. (*B*) Cell recovery after infection. Note the significant reduction in Venus-expressing cells after CDKI overexpression. (*C*) Appearance of GS cell colonies at 21 d postinfection. Whereas GS cells transduced with the control empty vector survived and showed fluorescence, Venus-positive cells were selectively lost after p21 overexpression, suggesting loss of SSCs. (*D*) Cell cycle analysis of Venus^{+} GS cells at 3 d postinfection. Note the p21-induced cell cycle arrest. (*E*) Real-time PCR analysis of p21 and p27 expression in GS cells. GS cells were cultured on laminin for 3 d under the indicated culture conditions. The values were normalized to Hprt1 expression, with expression levels in WT GS cells cultured without cytokines. E, EGF; F, bFGF; G, GDNF. Bar = 100 μm (*C*).

Table S1. Competition assay

Recipient ID	Period to first offspring (days) *	Periods of analysis (days)	Litter number	Transgenic offspring/total (%)
p21				
D	117	230	7	52/52 (100.0)
E	101	304	14	93/95 (97.9)
I	139	304	10	61/65 (93.8)
Total			31	206/212 (97.2)
p27				
H	89	195	7	10/49 (20.4)
U	153	205	5	2/35 (5.7)
V	124	169	3	0/14 (0.0)
Total			15	12/98 (12.2)

*Days from transplantation to the birth of first offspring are shown.

Table S2. Summary of serial transplantation

Donor mouse ID		No. of colonies dissected out *	No. of colonies serially transplanted †	No. of colonies regenerated ‡	Increase in colony number	Multiplication of colony §
WT						
1	R	10	7.5	18	10.5	24
	L	10	8.0	7	-1.0	9
2	R	13	10.4	15	4.6	14
	L	10	8.0	14	6.0	18
3	R	9	7.2	9	1.8	13
	L	12	9.6	21	11.4	22
4	R	7	5.6	11	5.4	20
	L	5	3.5	14	10.5	40
5	R	12	9.6	58	48.4	60
	L	12	9.6	45	35.4	47
6	R	6	4.8	12	7.2	25
	L	6	4.8	22	17.2	46
7	R	5	2.7	10	7.3	37
	L	12	6.0	21	15.0	35
p21 KO						
1	R	2	0.5	5	4.5	94
	L	4	3.2	4	0.8	13
2	R	3	2.4	10	7.6	42
	L	3	2.4	4	1.6	17
3	R	6	4.5	10	5.5	22
	L	3	2.3	1	1.3	4
4	R	9	6.8	12	5.2	18
	L	9	7.2	24	16.8	33
5	R	5	3.8	12	8.2	32
	L	7	5.6	7	1.4	13
6	R	10	7.5	12	4.5	16
	L	5	4.0	5	1.0	13
7	R	8	6.4	14	7.6	22
	L	5	4.0	13	9.0	33
p27 KO						
1	R	4	2.3	3	0.7	13
	L	5	3.3	0	-3.3	0
2	R	9	7.2	0	-7.2	0
	L	9	7.2	3	-4.2	4
3	R	11	8.8	3	-5.8	3
	L	6	4.8	3	-1.8	6
4	R	7	5.6	3	-2.6	5
	L	7	5.6	2	-3.6	4
5	R	8	6.4	4	-2.4	6
	L	7	5.6	8	2.4	14
6	R	3	2.4	4	1.6	17
	L	7	3.2	5	1.8	16
7	R	3	3.8	0	-3.8	0
	L	5	4.0	5	1.0	13

R, right testis, L, left testis.

*The number of germ cell colonies that were used for serial transplantation.

†The number of colonies transplanted into the next recipient mice, calculated by comparing the volume of cell suspension prepared and those actually introduced into the recipient testes.

‡The resultant colony number in the recipient mice in total.

§(Regenerated colony number)×10/number of colonies serially transplanted.

Table S3. PCR primers used for real-time PCR

Primer	Sequence
p21 Forward	5′-GCAGATCCACAGCGATATCC-3′
p21 Reverse	5′-CAACTGCTCACTGTCCACGG-3′
p27 Forward	5′-AAGGGCCAACAGAACAGAAG-3′
p27 Reverse	5′-GGATGTCCATTCAATGGAGTC-3′
E-cadherin Forward	5′-ACCGATTCAAGAAGCTGGC-3′
E-cadherin Reverse	5′-ACCATCCTAACACAGACAGTCC-3′
PLZF Forward	5′-CACACTCAAGAGCCACAAGC-3′
PLZF Reverse	5′-ATCATGGCCGAGTAGTCTCG-3′
Hprt1 Forward	5′-GCTGGTGAAAAGGACCTCT-3′
Hprt1 Reverse	5′-CACAGGACTAGAACACCTGC-3′

Table S4. PCR primers used for genotyping

Primer	Sequence
p21+116F	5′-AAGCCTTGATTCTGATGTGGGC-3′
p21−135	5′-TGACGAAGTCAAAGTTCCACCG-3′
Neo 19+	5′-GCTATCAGGACATAGCGTTGGC-3′
p27ex1-5′	5′-CCTGGAGCGGCTGGACGCCAGACA-3′
p27ex1-3′	5′-CACCAAATGCCGGTCCTCAGAGTT-3′
BK1	5′-GGCTATTGGCTCAAAACGAACCTC-3′
MK4	5′-ATGCTCCAGACTGCCTTGGGAAAA-3′

For genotyping of p21 KO mice, approximately 900 bp band is amplified with p21 + 116F and p21 − 135 primers, and approximately 759 bp is amplified with p21 + 116F and Neo 19+ primers. For genotyping of p27 KO mice, 350 bp band is amplified with p27ex1-5′ and p27ex1-3′ primers, and 380 bp band is amplified with BK1 and MK4 primers.