SUPPLEMENTARY MATERIAL

Supplementary Table 1: *Bmi-1*$^{-/-}$ neurospheres and uncultured progenitors from the CNS and PNS upregulate *p16*^{Ink4a} by quantitative PCR (see Supplementary fig. 4 for details).

		Fold change in *p16* (KO/WT)	Fold change in *p19* (KO/WT)
CNS, cultured	E15 CNS neurospheres	9.5±3.4	3.0
	P0 CNS neurospheres	6.4±2.6	1.7±0.2
	P30 CNS neurospheres	21.1±5.2	1.4±1.2
CNS, uncultured	E15 telencephalon	U in WT; U in KO	U in WT; U in KO
	P0 SVZ	U in WT; D in KO	U in WT; D in KO
	P30 SVZ	78.4±21.6	3.6±0.9
	Sorted P30 SVZ	14.4	1.9
	Sorted P30 SVZ $FSC^{hi}SSEA\text{-}1^{hi}CD24^{-/low}$	60.6	D in WT; D in KO*
PNS, cultured	E14 PNS neurospheres	5.3±0.3	2.7±0.3
	P0 PNS neurospheres	20±3	3.0±0.2
	P30 PNS neurospheres	9.0±6	1.5±0.6
PNS, uncultured	Uncultured P0 $p75^{+}$ cells	37	5
	Uncultured P30 $p75^{+}$ cells	25.0±12	U in WT; U in KO

Mean±SD for fold change values are presented when *p16*^{Ink4a} or *p19*^{Arf} could be detected in both *Bmi-1*$^{+/+}$ (WT) and *Bmi-1*$^{-/-}$ (KO) samples. In cases where *p16*^{Ink4a} or *p19*^{Arf} could not clearly be detected in one or both of the samples, results are indicated as Undetected (U) or Detected (D). Each data point represents 1-6 independent experiments. * Transcripts were detected at levels that were too low to calculate a meaningful ratio.

Supplementary Table 2: Genes expressed at higher levels in *Bmi-1*$^{-/-}$ neurospheres as compared to *Bmi-1*$^{+/+}$ neurospheres cultured from the P0 SVZ (CNS) and the P0 gut (PNS). Of 36,701 probe sets examined, we found 11 genes that were expressed at significantly higher levels in CNS and PNS *Bmi-1*$^{-/-}$ neurospheres (fold change (FC)>2, P<0.05) and that were also differentially expressed by qRT-PCR (FC>1.5) in independent samples of CNS and PNS *Bmi-1*$^{-/-}$ and *Bmi-1*$^{+/+}$ neurospheres. Note that the *Cdkn2a* probe set was complementary to the common domain of *p16*^{Ink4a} and *p19*^{Arf} and therefore did not distinguish between these transcripts.

			PNS neurospheres		CNS neurospheres	
Symbol	Gene Title	UniGene	Microarray FC (KO/WT)	qPCR KO/WT	Microarray FC (KO/WT)	qPCR KO/WT
Hoxc9	*Homeobox C9*	Mm.4765	16.6	220.9	2.5	27.2
Ccnd2	*Cyclin D2*	Mm.3141	15.3	60.0	2.7	2.2
Null	*RIKENcDNA9330107J05*	Mm.40831	6.7	7.1	2.1	1.7
Hoxd9	*Homeobox D9*	Mm.26544	6.2	5.3	4.3	12.6
Hoxd8	*Homeobox D8*	Mm.200521	5.1	6.3	5.8	255.9
Cdkn2a	*Cyclin-dep. kinase inhibitor 2A (p16/p19)*	Mm.4733	4.0		3.5	
Gas6	*Growth arrest specific 6*	Mm.3982	3.6	4.3	2.4	1.7
Cdkn1a	*Cyclin-dependent kinase inhibitor 1A (p21)*	Mm.34446	3.3	5.5	2.5	2.2
Vax2	*Ventral anterior homeobox containing gene 2*	Mm.57253	3.3	10.4	2.5	7.7
Pmaip1	*PMA-induced protein 1*	Mm.258086	2.8	3.8	2.8	2.1
Raet1c	*Retinoic acid early transcript gamma*	Mm.87808	2.2	7.7	2.7	6.1

Supplementary figure 1: *Bmi-1*-deficient mice exhibit a progressive growth retardation postnatally. Wild-type (+/+), heterozygous (+/-), and *Bmi-1*-deficient (-/-) mice appear grossly indistinguishable at E14.5 (**a**) and at birth (**b**). By postnatal day 30, the *Bmi-1*$^{-/-}$ mice were clearly growth retarded relative to their wild-type littermates, but still appeared normally proportioned as though there was a similar degree of growth retardation in many different tissues (**c**). The masses of E14.5 (**d**) and P0 (**e**) pups did not significantly differ between *Bmi-1*$^{+/+}$ and *Bmi-1*$^{-/-}$ genotypes, but at P30, *Bmi-1*$^{-/-}$ mice were significantly smaller (*, $p<0.01$) than their wild-type littermates (**f**).

Supplementary figure 2: CNS neurospheres and PNS neurospheres express *Bmi-1*. **a**, SYBR green fluorescence versus cycle number plot of *ß-actin* and *Bmi-1* transcripts from P30 *Bmi-1*$^{+/+}$ (WT) and *Bmi-1*$^{-/-}$ (KO) neurospheres (that had been cultured for 10 days) by LightCycler quantitative (real-time) RT-PCR. cDNA was random primed, and cDNA levels were normalized with respect to *ß-actin* expression. Cycle thresholds (C_T) for *Bmi-1* in *Bmi-1*$^{+/+}$ and *Bmi-1*$^{-/-}$ samples were 26.9 and >36 (below threshold) respectively. Note that beyond cycle 36, non-specific amplification products sometimes arise. Amplification efficiencies of *ß-actin* and *Bmi-1* primers were 95.2% and 91.9% respectively. **b**, Melting curve analysis indicated that a single product was amplified during the *Bmi-1* PCR reaction from the *Bmi-1*$^{+/+}$ sample. **c**, End-products of the qPCR reaction were separated on a 2% agarose gel to confirm the presence of a single band of the expected size for *ß-actin* (77bp) and *Bmi-1* (183 bp) in the *Bmi-1*$^{+/+}$ but not the *Bmi-1*$^{-/-}$ sample. **d,** SYBR green fluorescence versus cycle number plots of *ß-actin* and *Bmi-1* transcripts from PNS neurospheres cultured from P0 gut. Cycle thresholds (C_T) for *Bmi-1* were 28.5 for *Bmi-1*$^{+/+}$ and >36 (below threshold) for the *Bmi-1*$^{-/-}$ sample. **e,** Melting curve analysis

indicated that a single product was amplified during the *Bmi-1* qPCR reaction. **f,** End-products of the qPCR reactions were separated on 2% agarose gels to confirm the amplification of single bands of the expected size. **g**, microarray analysis of P0 PNS neurospheres indicated that *Bmi-1* was expressed in two replicate samples (signal intensities 8111 and 9474) as were positive control genes *ß-actin* and *GAPDH*, but not negative control genes *CD8* and *immunoglobulin heavy chain* (signal intensities below 100 were similar to background). Uncultured rat gut NCSCs that were purified by flow-cytometry as $p75^{+}\alpha_4^{+}$ cells [8] also expressed *Bmi-1* by PCR (data not shown).

Supplementary figure 3: *Bmi-1*-deficient CNS stem cells and NCSCs undergo multilineage differentiation. E14.5 telencephalon cells or postnatal day 0 (P0) or P30 lateral ventricle SVZ cells proliferated to form spherical colonies called neurospheres [28] when cultured at low density (2000 to 5000 cells per 35mm dish) under non-adherent conditions. Because neurospheres derive from a single cell and contain self-renewing multipotent progenitors, the neurosphere formation assay has been widely used to measure the frequency of cells capable of exhibiting stem cell activity in culture [16,28-30] [31,32]. *Bmi-1*$^{+/+}$ or *Bmi-1*$^{-/-}$ CNS progenitors (**a**) underwent multilineage differentiation in adherent cultures. CNS-derived colonies were considered multipotent if they contained neurons (ßIII-tubulin^{+}), and glia (GFAP^{+} astrocytes and/or O4^{+} oligodendrocytes). Note that the frequency of multipotent neurospheres in Figure 1 represents the percentage of cells that formed neurospheres multiplied by the percentage of neurospheres that formed neurons and glia when transferred to adherent cultures. 97% of *Bmi-1*$^{+/+}$ E14.5 neurospheres and 89% of *Bmi-1*$^{-/-}$ neurospheres formed neurons (Neuron-specific ßIII-tubulin^{+}) and glia (GFAP^{+} astrocytes and/or O4^{+} oligodendrocytes). CNS neurospheres did not appear to

adopt PNS identities as they failed to express peripherin upon differentiation (not shown). **b**, PNS neurospheres that formed from dissociated E14.5 gut tissue underwent multilineage differentiation to form neurons (peripherin$^+$), glia (GFAP$^+$) and myofibroblasts (smooth muscle actin$^+$; SMA). 94% of *Bmi-1*$^{+/+}$ PNS neurospheres and 92% of *Bmi-1*$^{-/-}$ neurospheres formed neurons, glia and myofibroblasts upon transfer to adherent cultures. PNS neurospheres did not appear to adopt CNS identities as they failed to generate O4$^+$ oligodendrocytes (data not shown).

Although gut NCSCs have previously been characterized based on their ability to form multilineage colonies in adherent cultures [9], the same gut NCSC population is able to form neurospheres in non-adherent cultures. Rat gut NCSCs have been isolated as p75$^+\alpha_4^+$ cells [9]. When these p75$^+\alpha_4^+$ NCSCs were cultured under non-adherent conditions, 28±5% survived to form neurospheres and 85% of those neurospheres were multipotent. Colonies that arose from p75$^+\alpha_4^+$ gut NCSCs in culture could be serially passaged as neurospheres and as adherent colonies irrespective of whether they were first cultured under adherent or non-adherent conditions (data not shown). Thus individual gut NCSCs appear to be capable of forming both neurospheres and adherent multilineage colonies.

Supplementary figure 4: *Bmi-1*-deficiency leads to the increased expression of the cyclin-dependent kinase inhibitor *p16*^{Ink4a} in cultured CNS stem cells and gut NCSCs. **a**, SYBR green fluorescence versus cycle number plot of *ß-actin* and *p16*^{Ink4a} transcripts from P30 *Bmi-1*$^{+/+}$ (WT) and *Bmi-1*$^{-/-}$ (KO) CNS neurospheres (that had been cultured for 10 days after dissociating cells from the SVZ) by quantitative (real-time) RT-PCR. RNA was purified from approximately 100 neurospheres for each sample, cDNA was random primed, and cDNA levels were normalized with respect to *ß-actin* expression. Cycle thresholds (C_T) for *p16*^{Ink4a} in *Bmi-1*$^{+/+}$ and

Bmi-1$^{-/-}$ samples were 30.7 and 27.1 respectively. The amplification efficiencies of *ß-actin* and *p16*^{Ink4a} were 95.2% and 98.6% respectively. Products of the qPCR reaction after 29 cycles were separated on a 2% agarose gel to confirm the presence of a single band of the expected size for *ß-actin* (77bp) and *p16*^{Ink4a} (89 bp). Based on the efficiency of amplification [33] (see Supplementary Methods) we calculated that *p16*^{Ink4a} was expressed at 17-fold higher levels in these *Bmi-1*$^{-/-}$ neurospheres. Using two different sets of primers against *p16*^{Ink4a} in multiple sets of samples, *Bmi-1*$^{-/-}$ P30 CNS neurospheres averaged 21.1-fold higher *p16*^{Ink4a} expression (Table 2). **b,** SYBR green fluorescence versus cycle number plots of *ß-actin* and *p19*^{Arf} transcripts from the same CNS neurospheres. Cycle thresholds (C_T) for *p19*^{Arf} were 26.4 for the *Bmi-1*$^{+/+}$ sample and 27.7 for the *Bmi-1*$^{-/-}$ sample. This suggested that these *Bmi-1*$^{-/-}$ and *Bmi-1*$^{+/+}$ neurospheres had similar levels of *p19*^{Arf} RNA expression. End-products of the qPCR reactions were separated on a 2% agarose gel to confirm the amplification of single bands of the expected size (142bp for *p19*^{Arf}). On average, *Bmi-1*$^{-/-}$ P30 CNS neurospheres averaged 1.4-fold higher *p19*^{Arf} expression (Table 2). **c**, SYBR green fluorescence versus cycle number plot of *ß-actin* and *p16*^{Ink4a} transcripts from P30 *Bmi-1*$^{+/+}$ (WT) and *Bmi-1*$^{-/-}$ (KO) PNS neurospheres (that had been cultured for 10 days after dissociating cells from the gut) by qPCR. Cycle thresholds (C_T) for *p16*^{Ink4a} in *Bmi-1*$^{+/+}$ and *Bmi-1*$^{-/-}$ samples were 27.7 and 24.0 respectively. Products of the qPCR reaction after 28 cycles were separated on a 2% agarose gel to confirm the presence of a single band of the expected size. Based on the efficiency of amplification [33] we calculated that *p16*^{Ink4a} was expressed at 15-fold higher levels in these *Bmi-1*$^{-/-}$ neurospheres. **d,** SYBR green fluorescence versus cycle number plots of *ß-actin* and *p19*^{Arf} transcripts from the same PNS neurospheres. Cycle thresholds (C_T) for *p19*^{Arf} were 25.9 for the *Bmi-1*$^{+/+}$ sample and 25.0 for the *Bmi-1*$^{-/-}$ sample indicating that *Bmi-1*$^{-/-}$ PNS neurospheres had 2-fold higher levels of *p19*^{Arf} expression.

End-products of the qPCR reactions were separated on 2% agarose gels to confirm the amplification of single bands of the expected size. Melting curves for each of these PCR reactions were consistent with the amplification of a single product (not shown). **e**, Uncultured P30 *Bmi-1*$^{-/-}$ CNS SVZ cells showed increased expression of p16^{Ink4a} protein. Cultured *Bmi-1*$^{-/-}$ CNS stem cells also exhibited an increase in p16^{Ink4a} expression (not shown). **f**, *Bmi-1*$^{-/-}$ PNS neurospheres (NS) showed increased expression of p16^{Ink4a} protein after 10 days in culture.

Supplementary figure 5: *p16*^{Ink4a} deficiency does not affect the proliferation of restricted neuronal and glial progenitors in culture. We counted the number of cells per CNS neuron-only colony (**a**), CNS glial-only colony (**b**), PNS glial-only colony (**c**), and the percentage of BrdU+ cells within PNS glial-only colonies (**d**). No significant differences were observed between *p16*$^{-/-}$ and *p16*$^{+/+}$ restricted progenitor colonies according to any of these criteria. The CNS and PNS restricted progenitors were derived from the same preparations of adult SVZ and adult gut, respectively, as the stem cells that were described in Figure 3 as being more proliferative in the absence of *p16*^{Ink4a}. The number of cells per CNS restricted progenitor colony was counted after 12 days in culture. The PNS glial-only colonies were counted after 10 days in culture. The frequency of CNS SVZ cells and PNS gut cells that formed N-only or G-only colonies also did not significantly differ between *p16*$^{-/-}$ and *p16*$^{+/+}$ mice (data not shown). We also compared the number of cells per PNS glial-only colony among P0 gut cells from progeny of *bmi-1*$^{+/-}$*p16*$^{+/-}$ matings (**e**). Even among these mice there was no significant effect of *Bmi-1* or *p16*^{Ink4a} deficiency on the number of cells per glial-only colony. There was also no difference in the frequency of cells that formed glial-only colonies between these samples or in the rate of BrdU incorporation into the glial-only colonies (data not shown). **f**, Western analysis for p16^{Ink4a}

and β-actin of cellular lysates corresponding to P0 gut cells cultured in adherent conditions for 10 days in the presence or absence of Nrg. In the absence of Nrg (No add), most colonies were multilineage NCSC colonies, but in the presence of Nrg, most colonies were glial-only (not shown). Note that $p16^{Ink4a}$ remained upregulated in *Bmi-1*$^{-/-}$ progenitors even after treatment with Nrg, despite the fact that *p16*^{Ink4a} deficiency did not affect the proliferation of these cells.

SUPPLEMENTARY METHODS

Isolation of CNS and PNS progenitors

In all cases, tissue was removed, and suspended after dissociation in staining medium: L15 medium containing 1 mg/ml BSA (Sigma A-3912, St. Louis, MO), 10 mM HEPES (pH 7.4), penicillin/streptomycin (BioWhittaker, Walkersville, MD), and 25 μg/mL deoxyribonuclease type 1 (DNAse1, Sigma D-4527). After centrifuging, the cells were triturated, filtered through nylon screen (45 μm, Sefar America, Kansas City, MO), counted by hemocytometer, and plated.

Embryonic CNS telencephalons were removed and dissociated for 2 min at 37˚C in 0.5 mg/ml DNAse1 (Sigma) in Ca, Mg-free HBSS, then quenched with staining medium. For postnatal CNS experiments, P0 SVZ was dissociated in trypsin/EDTA (BioWhittaker, diluted 1:4 in Ca, Mg-free HBSS) for 4 min at 37˚C, then quenched with staining medium. Adult SVZ was minced, then dissociated for 20 min at 37˚C in 0.025% trypsin/0.5mM EDTA (Calbiochem, San Diego, CA) plus 0.001% DNAse1 (Roche, Indianapolis, IN), then quenched with staining medium containing 0.014% soybean trypsin inhibitor (Sigma).

Embryonic gut tissue, including stomach, small intestine, and hindgut were dissected into ice cold PBS and dissociated by incubating for 4 min at 37°C in trypsin/EDTA (BioWhittaker,

product 17-161E, diluted 1:10 in Ca, Mg-free HBSS) plus 0.25 mg/ml type 4 collagenase (Worthington, Lakewood NJ). P0 and P30 gut outer muscle/plexus layers were minced, and dissociated for 15 min at 37°C in 0.025% trypsin/EDTA (Gibco 25300-054, Grand Island, NY) plus 1 mg/ml type 4 collagenase (Worthington) in Ca, Mg-free HBSS.

Cell culture

Adherent cultures of CNS and PNS progenitors were performed by sequentially coating 6-well plates (Corning Incorporated, Corning, NY) with 150 μg/ml poly-d-lysine (Biomedical Technologies, Stoughton, MA) and 0.15 mg/ml human fibronectin (Biomedical Technologies) as described [34]. In most cases the culture medium was based on a 5:3 mixture of DMEM-low:neurobasal medium, but for adult CNS progenitors DMEM:F12 was used. The medium was supplemented with 20 ng/ml recombinant human bFGF (R&D Systems, Minneapolis, MN), 1% N2 supplement (Gibco), 2% B27 supplement (Gibco), 50 μM 2-mercaptoethanol, and penicillin/streptomycin (Biowhittaker). CNS cultures also contained 20 ng/ml EGF (R&D Systems) and, in the case of P0 and E14.5 cells, also 10% chick embryo extract (CEE; prepared as described[34]). PNS cultures contained 15% CEE, 35 mg/ml (110 nM) retinoic acid (Sigma), and 20 ng/ml IGF1 (R&D Systems). In some of the experiments 65 ng/ml neuregulin (CeNeS, Cambridge, MA) or 50 ng/ml BMP4 (R&D Systems) were added to the cultures. All cultures were maintained at 37˚C in 6% CO_2/balance air.

Immunocytochemistry

CNS colonies were stained by incubating them in O4 antibody (1:800 ascites, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) for 30 min at room

temperature (RT) prior to fixation in acid ethanol (5% glacial acetic acid in 100% ethanol) for 20 min at –20°C. After blocking, the antibodies Tuj1 (1:500, Covance, Princeton, NJ) and GFAP (1:200, Sigma G-3893) were used to label neurons and astrocytes respectively. PNS adherent colonies and neurospheres replated into poly-d-lysine treated plates were fixed in acid ethanol for 20 min at –20°C, washed, blocked, and triply labeled for Peripherin (1:1000, Chemicon International AB1530), GFAP (1:200), and αSMA (1:200, Sigma A-2547). For the proliferation studies cells were fixed in 70% ethanol for 30 minutes at –20°C and stained with anti-BrdU antibody (1:100, Caltag, Burlingame, CA). For caspase-3 staining, plates were fixed for 10 min at RT in 4% paraformaldehyde, blocked, then stained with anti-activated caspase-3 antibody (1:1000, Pharmigen, San Diego, CA). In all cases, cells were counter stained for 10 min at RT with 10 μg/ml DAPI (Sigma D-8417) to visualize nuclei.

Tissue fixation and immunohistochemistry

Mice were sacrificed two to three hours after BrdU injection, their brains fixed in 4% paraformaldehyde overnight, then cryoprotected in 15% sucrose, embedded in 7.5% gelatin/15% sucrose, and flash frozen. 10-12 μm sections were cut on a Leica cryostat. For detection of BrdU in the CNS tissue sections, DNA was first denatured in 2M HCl for 30 min at RT and neutralized with 0.1M Sodium Borate before blocking. All sections were pre-blocked for at least 1 hr at RT in goat serum solution (GSS) containing 5% goat serum, 1% BSA, and 0.3% Triton X-100 (Sigma). Primary antibodies (diluted in GSS) were incubated overnight at 4˚C, followed by secondary antibody for 3-4 hrs at RT. Slides were counter stained in 2.5μg/ml DAPI for 10 min at RT, then mounted using ProLong antifade solution (Molecular Probes, Eugene, OR).

Antibodies used included rabbit anti-activated caspase-3 (1:1000, Pharmigen), and rat anti-BrdU (1:200, Accurate Chemical, Westbury, NY).

Quantitative PCR

Aliquots of about 100 neurospheres were collected after 7-12 days in culture, and RNA was extracted using TRIZOL reagent (Invitrogen, Carlsbad, CA). Reverse transcription was performed using Superscript II (Invitrogen) and random primers, and cDNA was further purified by precipitation. cDNA was diluted so that the *ß-actin* content was similar for wild-type and knockout samples and amplified between 16 and 20 cycles by qPCR. qPCR was performed on a LightCycler (Roche) using a DNA Master FastStart SYBR Green Kit (Roche). Efficiencies were calculated for each set of primers using a standard curve generated by plotting cycle threshold versus log fluorescence, where $E = 10^{-1/slope}-1$. Fold-changes between wild type (WT) and knockout (KO) for each target gene were calculated using *ß-actin* as the reference gene according to the formula: Fold-change= $(1+E_{target})^{\Delta CT,target(WT-KO)}/(1+E_{ref})^{\Delta CT,ref(WT-KO)}$ (ref[33]). When no product was amplified within 36 rounds of PCR or when a late-amplifying product did not appear to be specific based on the melting curve, the transcript was considered undetectable.

Western blots

Cells were washed with phosphate-buffered saline (PBS), lysed in Laemmli sample buffer (Bio-Rad), and analyzed immediately or frozen at –80°C. For the analysis, proteins were separated in 12% denaturing SDS-PAGE gels and transferred to PVDF membranes (Bio-Rad). The membranes were blocked, incubated with primary and secondary antibodies, and washed following standard procedures. Secondary antibodies were detected by chemiluminescence (ECL

and ECL Plus; Amersham-Pharmacia). Primary antibodies were mouse monoclonal anti-ß-actin (Ab-1; Oncogene), and rabbit polyclonal anti-p16 (M-156; Santa Cruz Biotechnology).

PCR Primers

Two sets of primers were designed against *p16*^{Ink4a}: sense, 5'CGAACTCTTTCGGTCGTACCC-3'; antisense, 5'-CGAATCTGCACCGTAGTTGAGC-3'. These primers amplified an 89 nucleotide product that spanned an intron and that was identical to *p16*^{Ink4a} upon sequencing. A second set of primers was also used in some experiments to amplify *p16*^{Ink4a}: sense, 5'CATCTGGAGCAGCATGGAGTC-3'; antisense, 5'-GGGTACGACCGAAAGAGTTCG-3'. These primers generated a 128 nucleotide product that was identical to *p16*^{Ink4a} upon sequencing. Primers that amplified *p19*^{Arf} were: sense, 5'GTTCTTGGTCACTGTGAGGATTCAG-3' and antisense, 5'-CCATCATCATCACCTGGTCCAG-3'. These primers amplified a 192 nucleotide product that spanned an intron and that was identical to *p19*^{Arf} upon sequencing. *p16*^{ink4a} genotyping was performed by PCR as described [13]. *Bmi-1* genotyping was performed by PCR. The oligonucleotides 5'-CGCCGTGCACAGGGTGTCACGTTGCAAGAC -3' and 5'-CAAGCCAACCACGGCCTCCAGAAG-3' were used to detect the presence or absence of the hygromycin cassette, and the oligonucleotides 5'-AGCAGCAATGACTGTGATGCACTTGAG-3' and 5'-GCTCTCCAGCATTCGTCAGTCCATCCC-3' were used to detect wild-type *Bmi-1*.

RNA amplification for microarray analysis

Cultured CNS neurosphere cells derived from three wild type and two *Bmi-1*$^{-/-}$ mice were pipetted into Trizol with 250µg/ml glycogen (Roche Diagnostic Corporation, Indianapolis IN).

RNA was extracted following the manufacturer's instructions. The extracted RNA was treated for 30min at 37°C with 2µl of RNase-free DNase1 (2U/µl; Ambion, Austin TX) in the presence of 2µl of RNase inhibitor (10U/µl) (Invitrogen). The RNA was then purified with RNeasy Mini Kit (Qiagen, Valencia CA) according to the manufacture's instructions and washed 3 times with 500µl of RNase-free water in a Microcon YM-100 (Millipore, Bedford MA). 15µg of total RNA from each sample were amplified by T7 RNA polymerase. After adding 1µg T7-d(T)24 primer (containing a T7 RNA polymerase binding sequence; 5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG(T)24,; Proligo, Boulder CO), the RNA was dried down to 10µl. The first strand synthesis, second strand synthesis, in vitro transcription and purification of complementary RNA (cRNA) were performed according to the Affymetrix protocol (Affymetrix, Santa Clara CA).

In the case of cultured PNS neurospheres, we obtained approximately 400ng total RNA from neurospheres cultured from the guts of two wild type and two *Bmi-1*$^{-/-}$ mice. RNA was amplified through two consecutive rounds of amplification using a modified version of the method of Baugh et al. [35]. The extracted RNA with 0.025µg of T7-d(T)24 primer was dried to 2.5µl. First strand synthesis, second strand synthesis, in vitro transcription and purification of complementary RNA (cRNA) were performed according to the Affymetrix protocol except that the reaction volume of first-strand synthesis and second strand synthesis was one-fourth the scale of the single round RNA amplification method (until making double stranded cDNA). cDNA was extracted with phenol-chloroform and precipitated with 20µg glycogen. After resuspending with 14µl of RNase-free water, 4µl of 10X reaction buffer (Ampliscribe™ T7 High yield Transcription Kit; Epicentre, Madison WI), 12µl of 25mM NTP (Promega, Madison WI), 4µl of 0.1MDTT, 2µl of RNase inhibitor, and 4µl of T7 RNA polymerase (80U/µl) (Promega), the

mixture was incubated at 42°C for 6h. The resulting cRNA was washed three times in a microcon-100 column, collected and dried to 4μl. To begin the second round of amplification, 4μl of cRNA was mixed with 1μl of 0.15μg/μl random hexamers (Roche Diagnostic Corporation) and the mixture was incubated at 70°C for 10min, chilled on ice and followed by incubation at room temperature for 10min. Then 2μl of 5Xfirst strand synthesis buffer, 1μl of 0.1MDTT, 1μl of 10mMdNTP, 0.5μl of RNase inhibitor and 0.5μl of PowerScript Reverse Transcriptase (Clonetech, Palo Alto CA) were added and incubated at 37°C for 2h. Subsequently, 1μl of RNaseH (2U/μl) (Invitrogen) was added and incubated at 37°C for 20min followed by heat-inactivation at 95°C for 2min.

For second strand synthesis, 1μl of 0.1μg/μl T7-d(T)24 primer was added and the mixture was incubated at 70°C for 5min and 42°C for 10min. Next, 53μl of RNase-free water, 7.5μl of 10Xsecond strand synthesis buffer, 1.5μl of 10mM dNTPs, 2μl of DNA Polymerase1 (10U/μl) (New England BioLabs, Beverly MA) and 0.5μl of RNaseH (2U/μl) were added and the mixture was incubated at 16°C for 2h. 2μl of T4 DNA Polymerase (5U/μl) (Invitrogen) was added and the mixture was incubated for an additional 15min at 16°C. The resulting cDNA was extracted with phenol-chloroform and precipitated with 20μg glycogen. The double stranded cDNA was resuspended with 22μl RNase-free water and transcribed to cRNA with the biotin labeling kit (BioArray Highyield RNA transcript labeling kit (T7), Enzo Diagnostics, Farmingdale NY). cRNA was purified using the RNeasy Mini Kit. 60~80μg of biotinylated cRNA were obtained from RNA amplification.

Hybridization and data analysis

15μg cRNA were fragmented and hybridized per chip to Mouse U74 whole genome chips (Chips A, B and C; Affymetrix) [36]. The chips were hybridized and scanned according to the

manufacturer's instructions. Signal intensities were read and processed for analysis using methods that have been described previously [37,38]. To measure fold changes between two cells, we set all negative signal intensity values or values less than 100 to 100. The statistical significance of differences in signal intensity for each probe set were evaluated by student's T-test using the logarithmic values.

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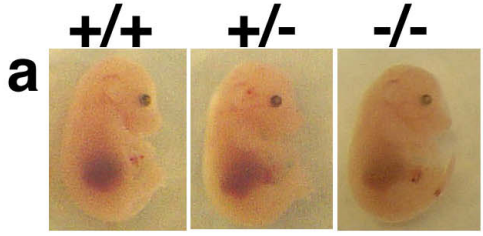
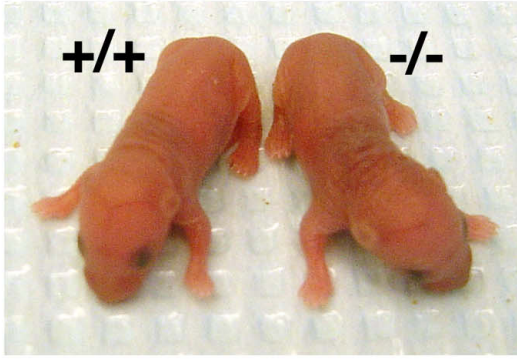
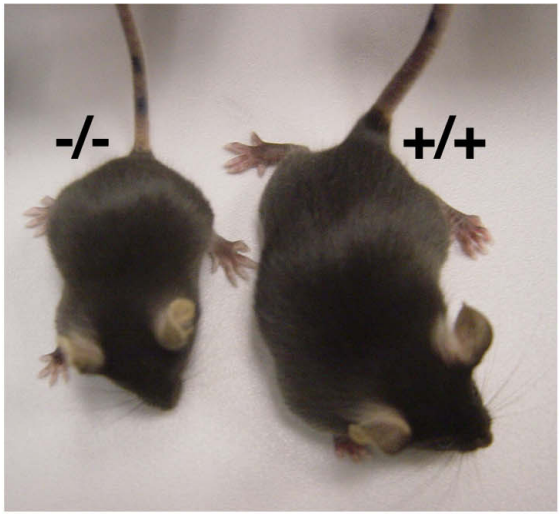
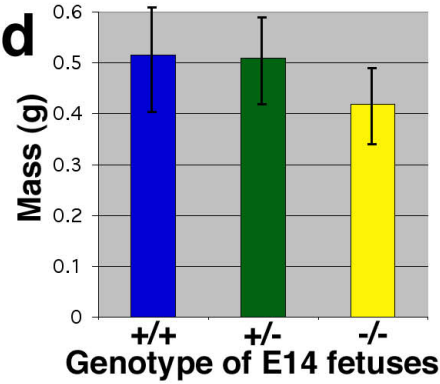
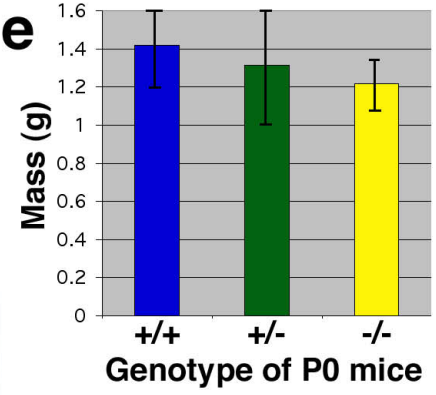
Supplementary figure 1

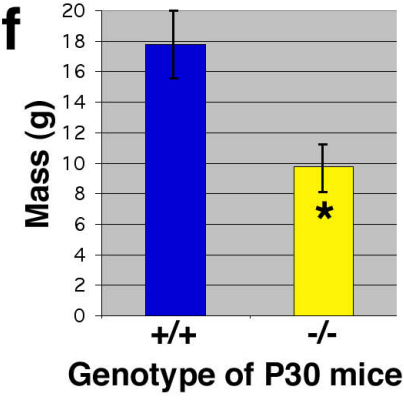






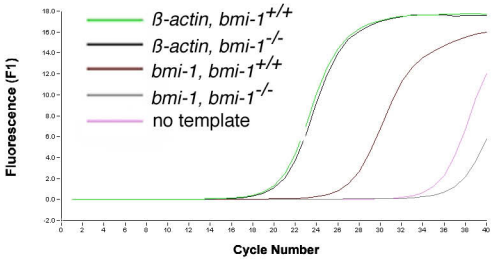






Supplementary figure 2

a. qPCR from CNS neurospheres



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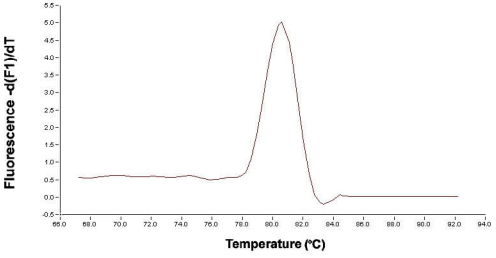

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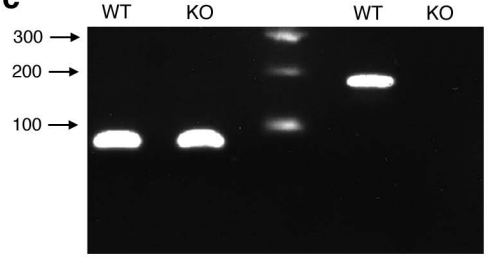
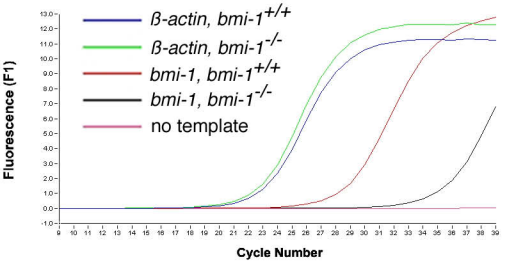


d. qPCR from PNS neurospheres



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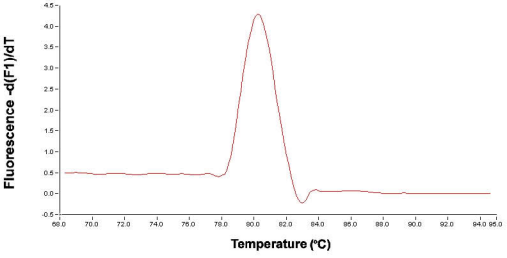

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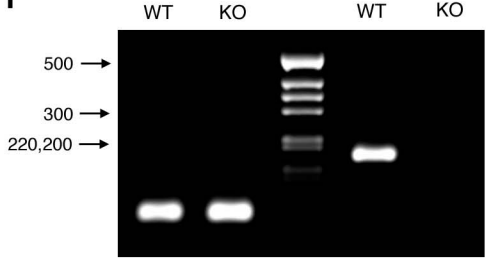
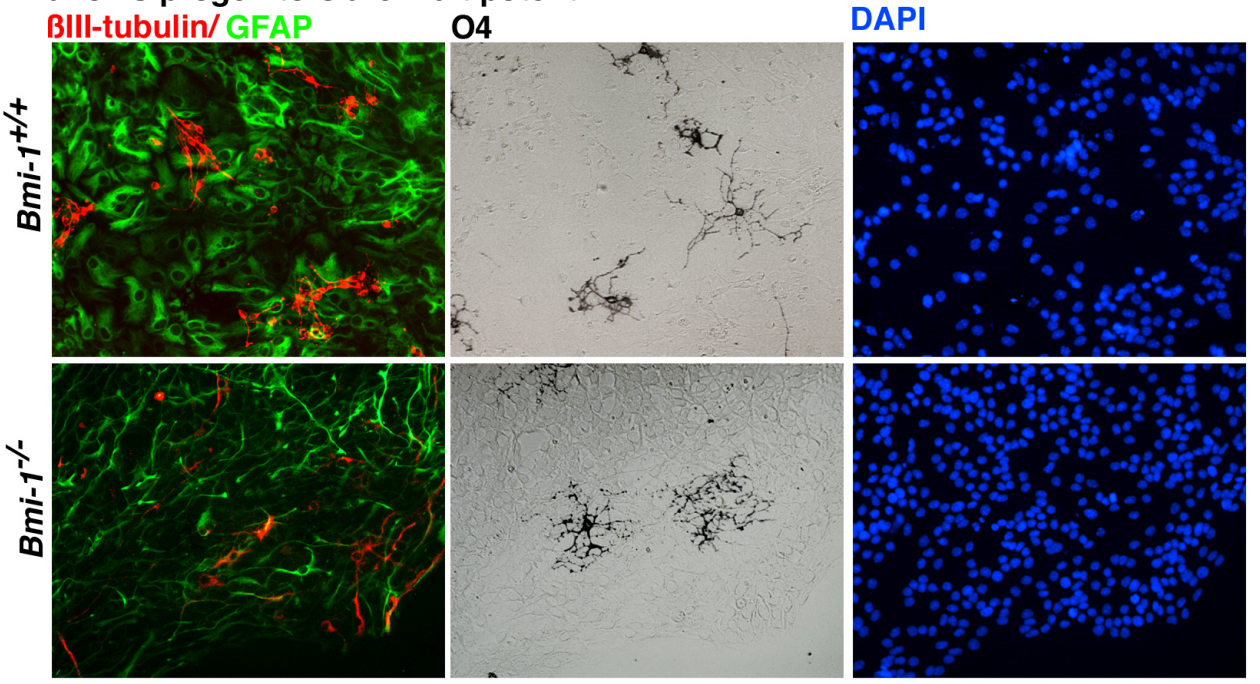


g. microarray analysis of PNS neurospheres

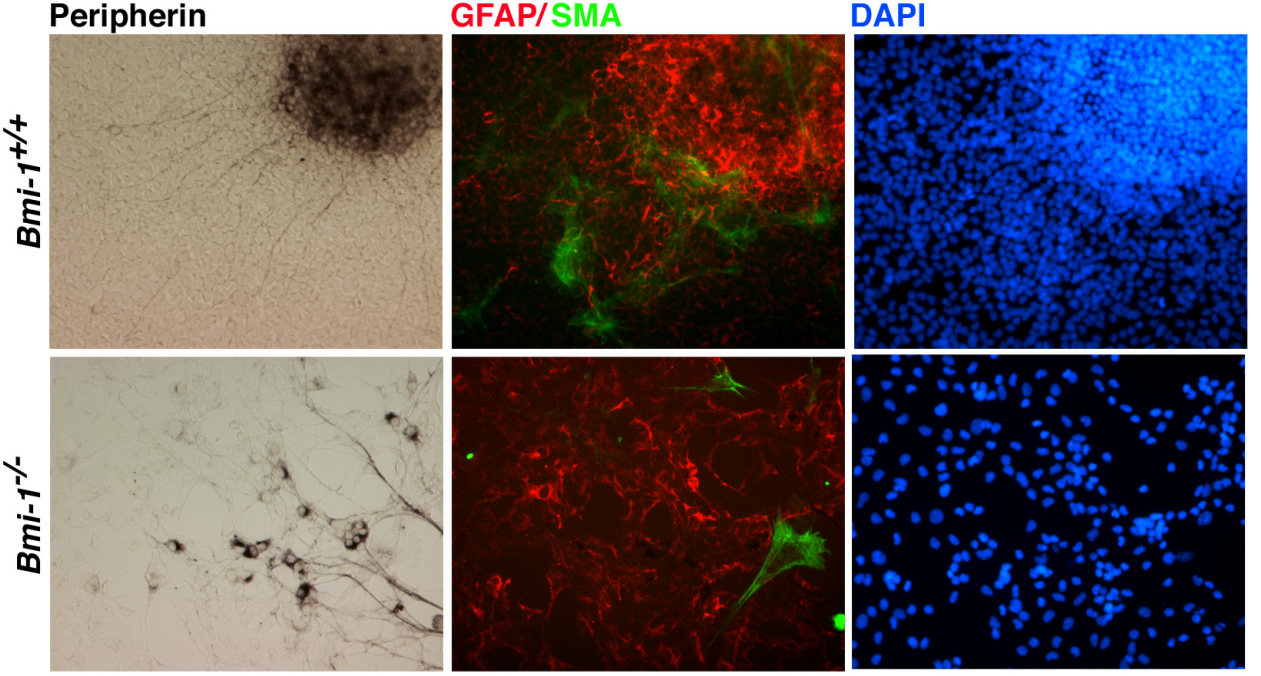
Gene Title	Probe-set Name	Gene Symbol	Unigene ID	WT1	WT2	Present Call
ß-actin	AFFX-b-ActinMur/M12481_3_at	Actb	Mm.297	52212	49426	2
GAPDH	AFFX-GapdhMur/M32599_3_at	Gapd	Mm.5289	19677	22013	2
CD8	102975_at	Cd8a	Mm.1858	-16	-1	0
Ig heavy chain Ia	99829_at	Igh-Ia	Mm.615	9	42	0
BMI-1	101475_at	Bmi1	Mm.7719	8111	9474	2

Supplementary figure 3

a. CNS progenitors are multipotent

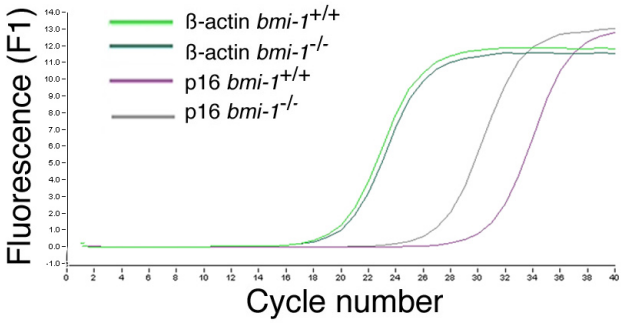


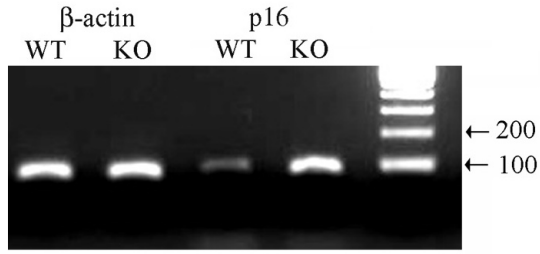
b. PNS neurospheres are multipotent



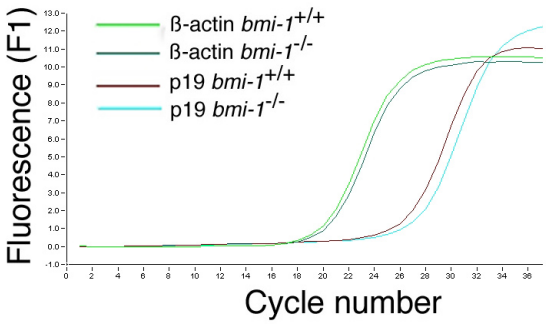
Supplementary figure 4

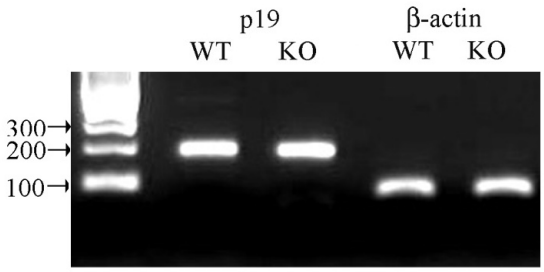
a. *p16^{Ink4a}* expression in CNS neurospheres



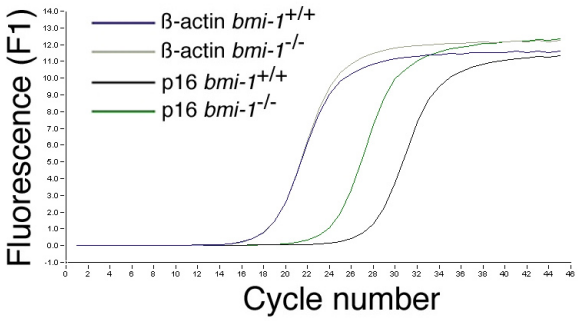


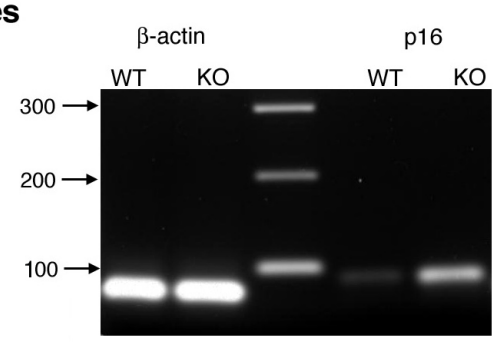
b. *p19^{Arf}* expression in CNS neurospheres



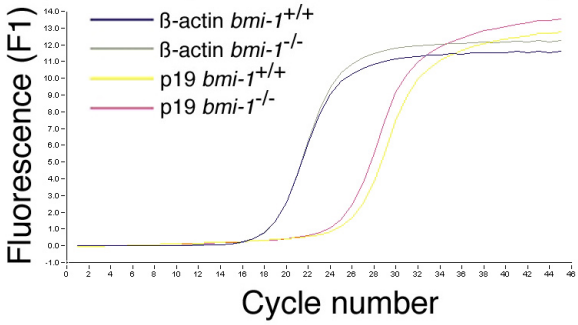


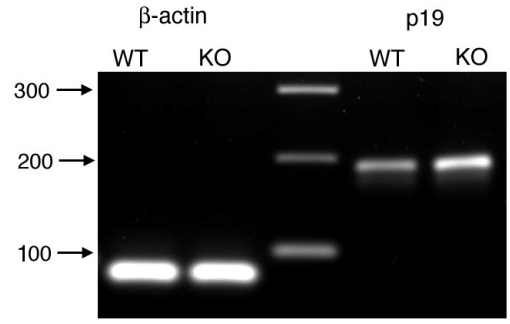
c. *p16^{Ink4a}* expression in PNS neurospheres





d. *p19^{Arf}* expression in PNS neurospheres





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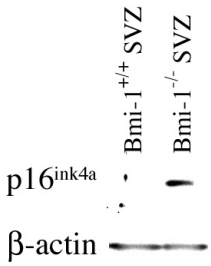

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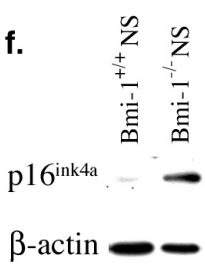

Supplementary Figure 5