SUPPLEMENTARY FIGURES

Supplementary Figure 1: *Prdm16* is expressed by neural stem/progenitor cells in the ventricular zone of the lateral ventricle of newborn mice. **a-d**) Antibody staining for ß-galactosidase indicated that *Prdm16* was expressed in the ventricular zone (VZ) around the lateral ventricle of newborn mice in a pattern that overlaps staining for the stem/progenitor cell marker Nestin (scale bar for **a** and **c** is 250μm, scale bar for **b** and **d** is 25μm).

Supplementary Figure 2: *Prdm16* is expressed by neural crest stem cells in the newborn gut and required for their self-renewal. a) Neural crest stem cell (NCSC) neurospheres were cultured from the outer muscle/plexus layers of the guts of *Prdm16*$^{+/+}$ (left) and *Prdm16*$^{LacZ/LacZ}$ (right) mice, then stained for ß-galactosidase activity using X-gal (blue). No ß-galactosidase staining was detected in *Prdm16*$^{+/+}$ neurospheres but nearly all *Prdm16*$^{LacZ/LacZ}$ neurospheres stained strongly for ß-galactosidase activity. **b**) Gut NCSCs can be isolated by flow-cytometry as p75+CD49b+ cells (see P5 box). Unfractionated outer muscle/plexus layer gut cells or p75+CD49b+ gut cells were stained with FDG to assess ß-galactosidase activity. 2.5-3.6% background staining was observed in *Prdm16*$^{+/+}$ cells (left). A minority of unfractionated outer muscle/plexus layer gut cells and a majority of p75+CD49b+ gut cells from *Prdm16*$^{LacZ/LacZ}$ mice stained positively for ß-galactosidase (right; FDG+). **c**) A significantly lower percentage of cells from the gut outer muscle/plexus layer of *Prdm16*$^{LacZ/LacZ}$ mice formed multipotent neurospheres in culture as compared to cells from littermate controls. **d**) The diameter of *Prdm16*$^{LacZ/LacZ}$ and *Prdm16*$^{LacZ/+}$ neurospheres was significantly decreased relative to *Prdm16*$^{+/+}$ neurospheres. The number of independent experiments is indicated in each panel, error bars always represent SD.

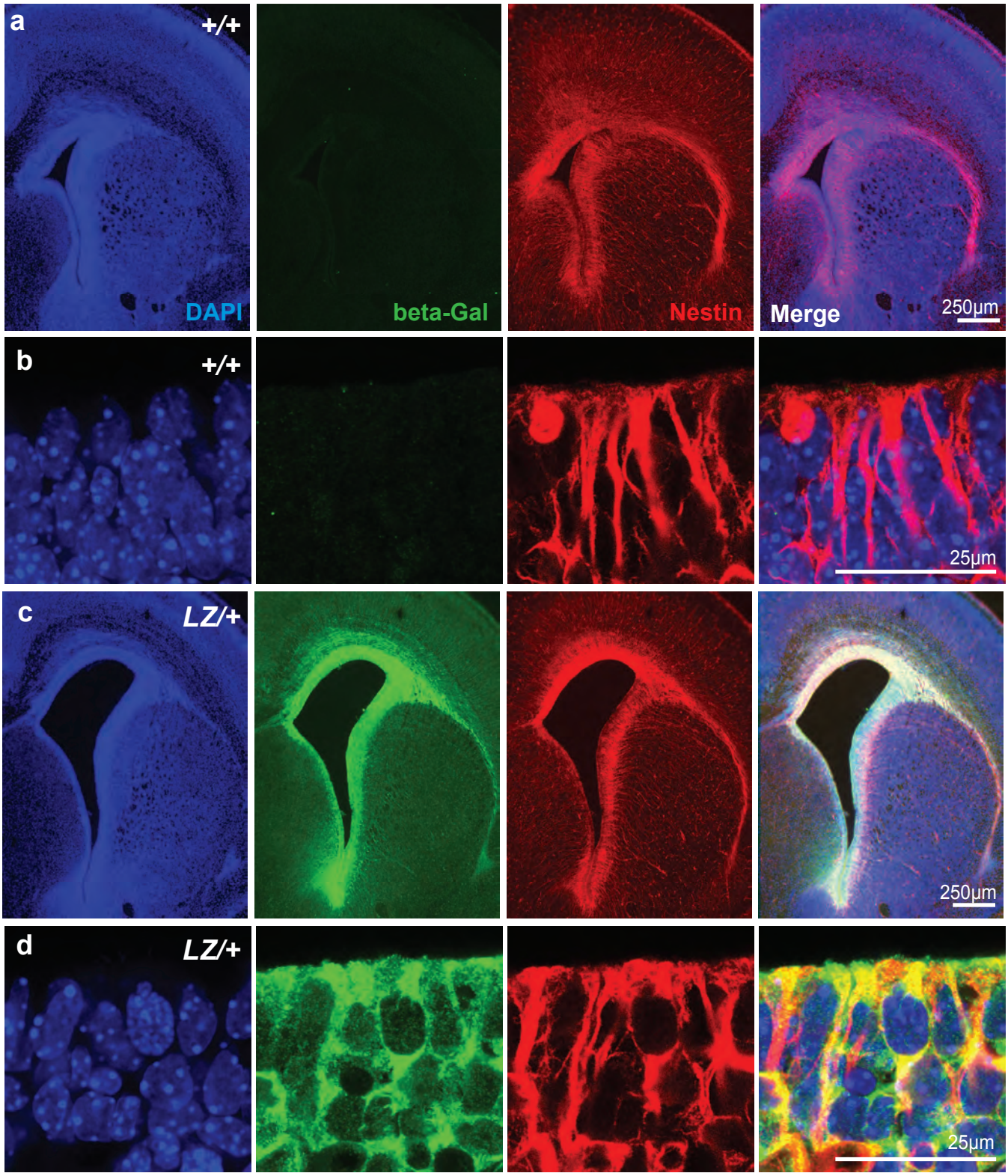
Supplementary Figure 3: HSCs were depleted in the spleens of newborn *Prdm16*$^{LacZ/LacZ}$ mice but differentiated cells were not. a-b) Newborn *Prdm16*$^{+/+}$, *Prdm16*$^{LacZ/+}$ and *Prdm16*$^{LacZ/LacZ}$ mice were born at the expected Mendelian frequency **c**) The spleens of newborn *Prdm16*$^{LacZ/LacZ}$ mice had normal frequencies of mature hematopoietic cells. No significant differences were detected in the frequencies of B (B220+), T (CD3+), erythroid (Ter119+), or myeloid (Mac-1+Gr-1+) lineage cells. Since spleen cellularity was also normal (Fig. 2a), the absolute numbers of differentiated cells was normal in the spleens of *Prdm16*$^{LacZ/LacZ}$ mice. Data are from at least 5 mice per genotype from at least 3 independent experiments. Error bars always represent SD. **d**) The spleens of newborn *Prdm16*$^{LacZ/+}$ and *Prdm16*$^{LacZ/LacZ}$ mice

contained significantly fewer $CD150^{+}CD48^{-}CD41^{-}Sca1^{+}c\text{-}kit^{+}$ HSCs compared to *Prdm16*$^{+/+}$ mice. The number of mice per genotype is indicated in the legend of panel **c** and in bars in panel **d**. Error bars always represent SD. **e)** The gene expression profiles of VZ cells from newborn *Prdm16*$^{+/+}$, *Prdm16*$^{LacZ/+}$, and *Prdm16*$^{LacZ/LacZ}$ mice were compared by microarray. The list shows all genes that were significantly ($p<0.05$) increased in expression within *Prdm16*$^{LacZ/LacZ}$ VZ cells as compared to *Prdm16*$^{+/+}$ or *Prdm16*$^{LacZ/+}$ cells (by at least 2.2 fold between *Prdm16*$^{+/+}$ and *Prdm16*$^{LacZ/LacZ}$ VZ and at least 1.8 fold between *Prdm16*$^{LacZ/+}$ and *Prdm16*$^{LacZ/LacZ}$). Genes that decreased in expression are shown in Figure 4a. Asterisks indicate genes associated with ROS regulation or response to oxidative stress. Differential expression was confirmed by qPCR in 3 independent samples/genotype.

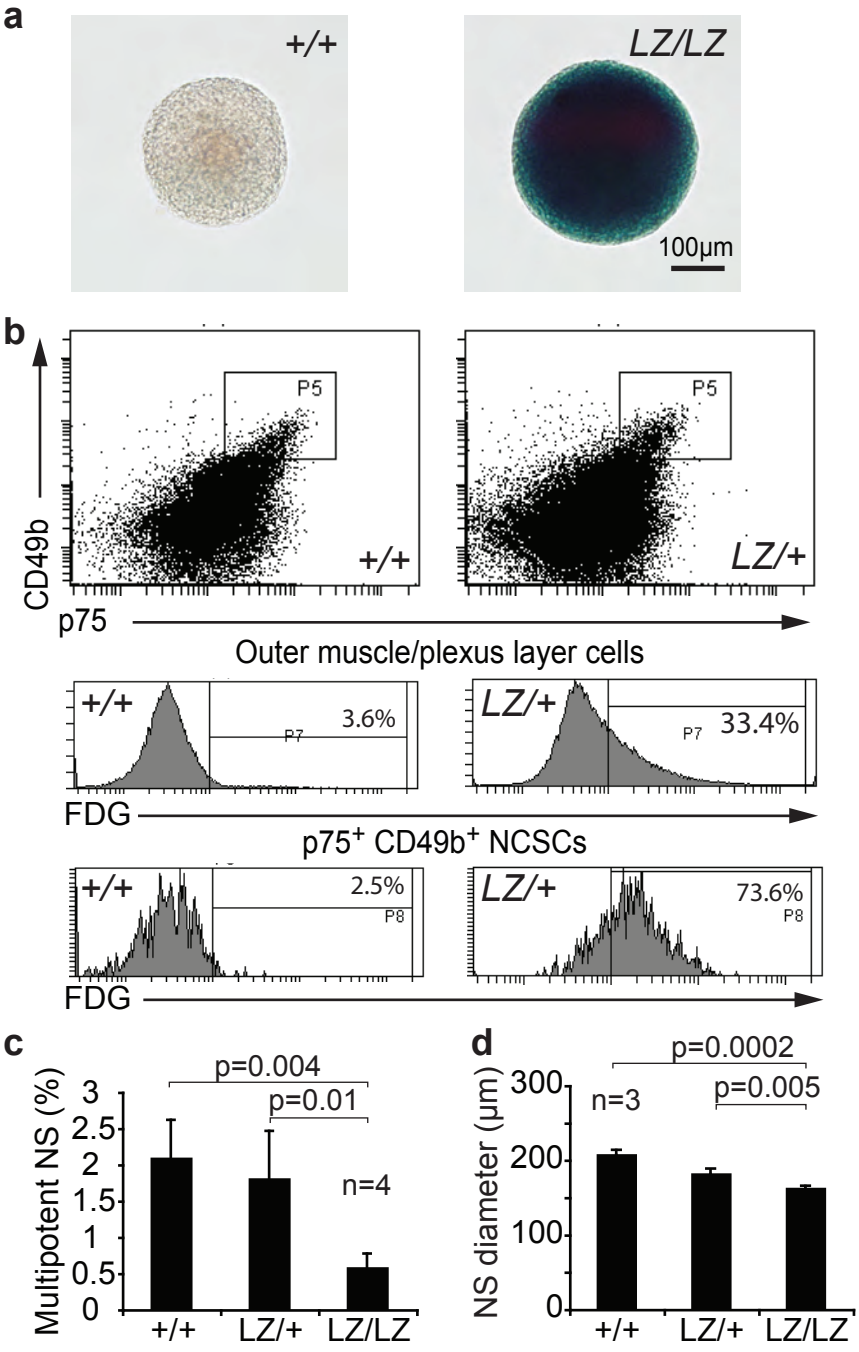
Supplementary Figure 4: ROS, mitochondrial mass, and mitochondrial membrane potential in newborn (P0) stem/progenitor cells from the hematopoietic and nervous systems of *Prdm16*$^{+/+}$, *Prdm16*$^{LacZ/+}$ and *Prdm16*$^{LacZ/LacZ}$ mice. a-b) *Prdm16* deficiency significantly reduced DCFDA staining in newborn c-kit+Sca-1+ cells as assessed by mean (**a**) or median (**b**) fluorescence (the total number of mice per treatment is indicated under each bar; each panel reflects at least 3 independent experiments). Newborn (P0) *Prdm16*$^{LacZ/LacZ}$ lateral ventricle VZ cells (**c-d**) and unfractionated liver or $Sca1^{+}c\text{-}kit^{+}$ hematopoietic cells (**e-f**) were assessed for mitochondrial mass using Mitotracker (**c,e**) and mitochondrial membrane potential using TMRM (**d,f**). No changes in mitochondrial mass or membrane potential were detected in neonatal VZ cells (data are from 2 *Prdm16*$^{+/+}$, 5 *Prdm16*$^{LacZ/+}$ and 5 *Prdm16*$^{LacZ/LacZ}$ mice). **c**) No significant change in mitochondrial mass was detected in P0 liver or $Sca1^{+}c\text{-}kit^{+}$ cells. A significant reduction in mitochondrial membrane potential was observed in *Prdm16*$^{LacZ/+}$ and *Prdm16*$^{LacZ/LacZ}$ hematopoietic cells from newborn liver. This trend toward reduced mitochondrial membrane potential could contribute to the reduced ROS levels in *Prdm16* deficient

hematopoietic stem/progenitor cells. (*, P<0.05; **, P<0.01; ***, P<0.001, error bars always represent SD).

Supplemental Figure 1

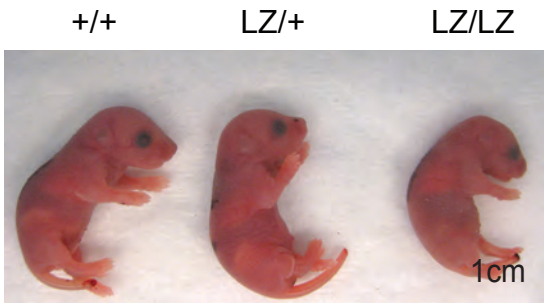


Supplemental Figure 2



Supplemental Figure 3

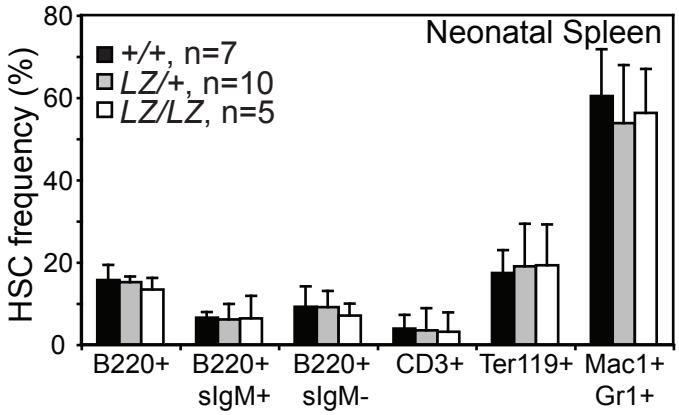
a



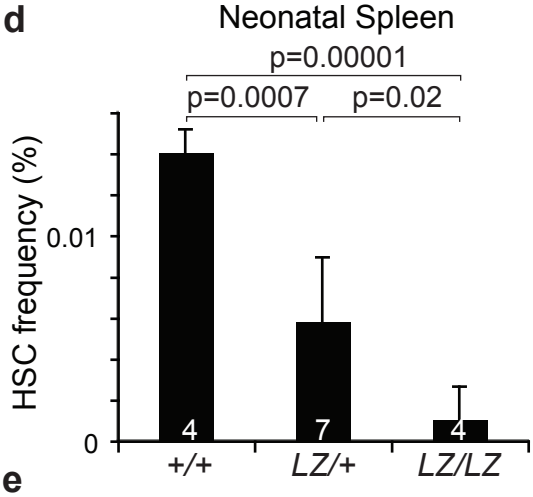
b

Litter	+/+	LZ/+	LZ/LZ
1	1	5	2
3	2	4	2
4	2	5	2
5	2	4	0
6	2	6	1
7	3	5	1
8	2	3	1
9	2	3	2
10	1	2	3
11	1	3	2
12	1	6	2
13	1	3	3
Sum	20	49	21
Percent	22	54	23

c



d



e

Symbol	Description	Microarray KO/WT	Microarray KO/HET	qRT PCR KO/WT	qRT PCR KO/HET
Has2	hyaluronan synthase 2	2.3	1.8	20±11	10±8
Enpp2 *	ectonucleotide pyrophosphatase/phosphodiesterase 2	2.4	2.2	3.0±0.1	2.9±0.6
Pdzrn3	PDZ domain containing RING finger 3	2.6	2.2	ND	ND
Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	2.6	1.8	6.9±2.4	4.1±1.1
Ptx3 *	pentraxin related gene	2.7	2.1	11±0	7.9±2.9
Moxd1	monooxygenase, DBH-like 1	3.7	3.4	ND	ND

Supplemental Figure 4