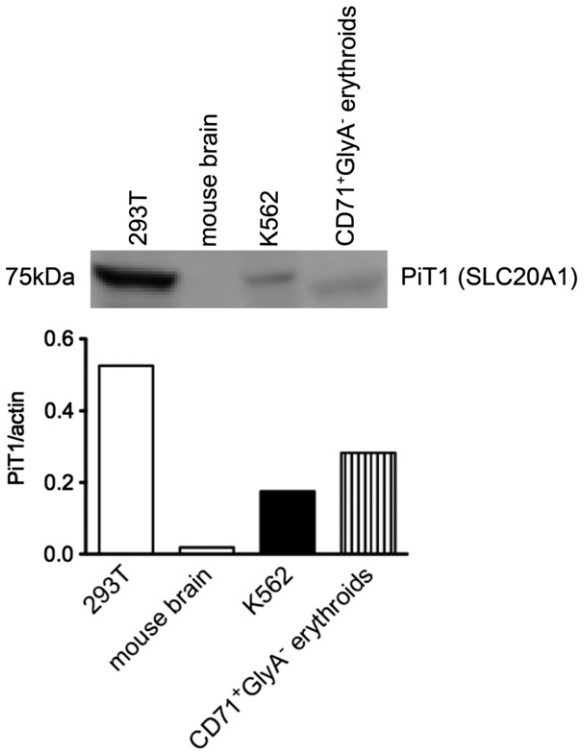
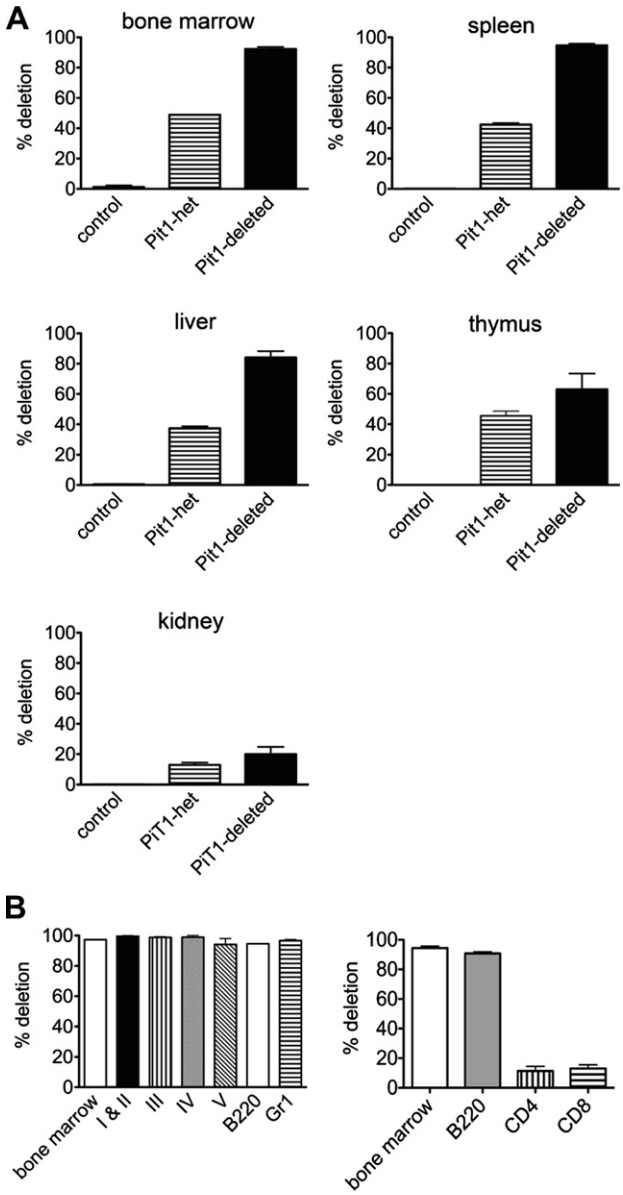
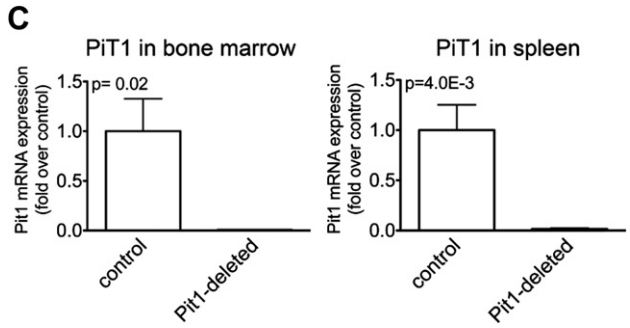


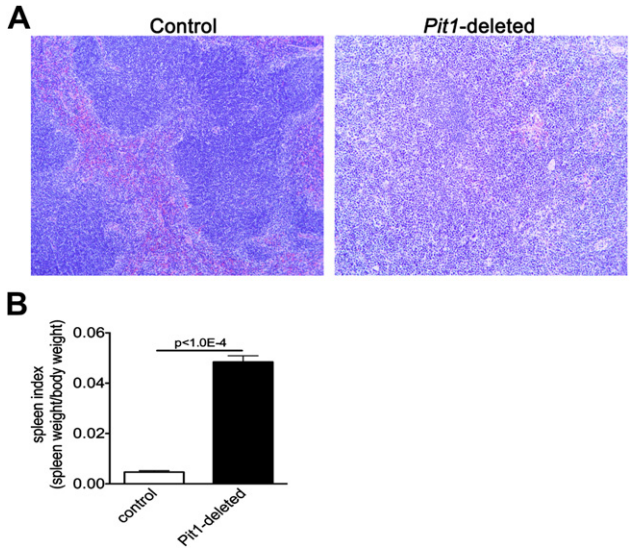
Supplementary Figure E1. SLC20A1 (PiT1) is expressed in human erythroid cells. Western blot of SLC20A1 in the human embryonic kidney cell line, 293T, the erythromyeloblastoid leukemia cell line, K562, and primary cultured human erythroid precursors ($CD71^+$glycophorin A^- cells). $CD71^+$glycophorin A^- cells were generated by erythroid culture of peripheral blood $CD34^+$ cells for 6 days. We are unable to detect PiT1 protein expression in mouse brain, which has high *Pit1* mRNA expression (data not shown), using an antibody generated against an immunogen with 100% sequence identity between murine and human PiT1. In our hands, no currently available commercial antibody or our own in-house antibody detects PiT1 in primary murine cells. Band intensities were normalized to β-actin (graph).



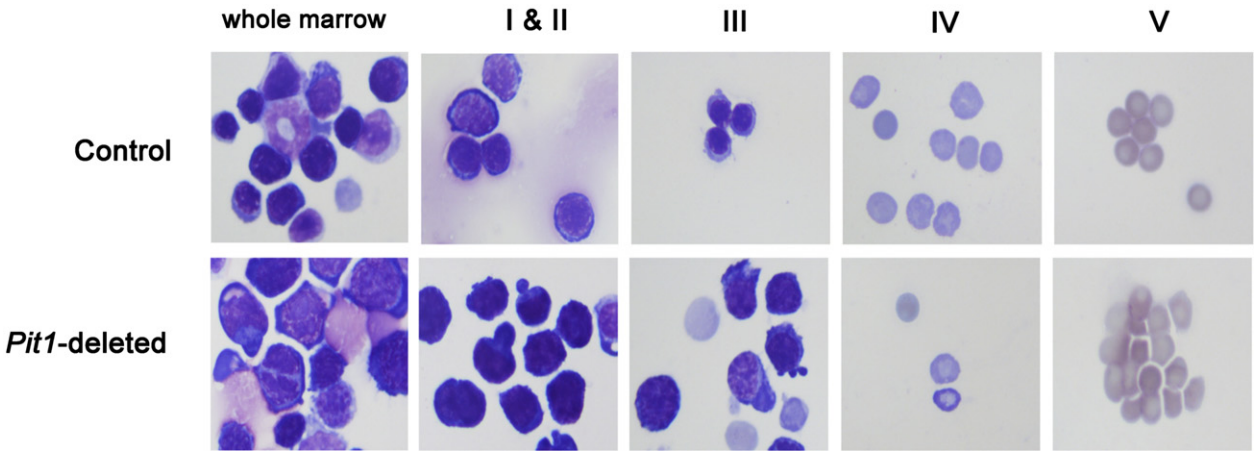
Supplementary Figure E2. Percent deletion of *Pit1* floxed-allele and expression of Pit1 mRNA in hematopoietic tissues from control and mutant mice. Tissues were harvested from 4–5-week-old mice, 3–4 weeks after deletion of the floxed allele. **(A)** Percent deletion of the *Pit1* floxed allele in whole bone marrow, spleen, liver, thymus, and kidney. Bone marrow, liver, kidney: control mice, n = 6; *Pit1*-heterozygously deleted mice (*Pit1*-het), n = 4; *Pit1*-deleted mice, n = 5. Spleen: control mice, n = 6; *Pit1*-het, n = 4; *Pit1*-deleted mice, n = 4. Thymus: control mice, *Pit1*-het, and *Pit1*-deleted mice, n = 5. **(B)** Percent deletion of the *Pit1* floxed-allele in whole bone marrow and flow cytometrically sorted bone marrow (left) and peripheral blood (right) populations. Bone marrow and sorted marrow populations (left, see Fig. 3B legend for details): n = 2 samples, each sample containing pooled marrow from 3 male or 2 female *Pit1*-deleted mice; $B220^+$ lymphocytes: n = 3 *Pit1*-deleted mice. Bone marrow and sorted peripheral blood populations (right): n = 6 *Pit1*-deleted mice. Percent deletion was measured by genomic quantitative real-time polymerase chain reaction (PCR). Sample PCRs were performed in triplicate. Percent deletion was quantified using an external standard encoding for both target sequences (wild type and *Pit1*-deleted alleles) and calculated as the ratio of deleted copy number to wild type allele plus deleted allele copy number. All data represent mean ± SEM, two-tailed Student *t* test.



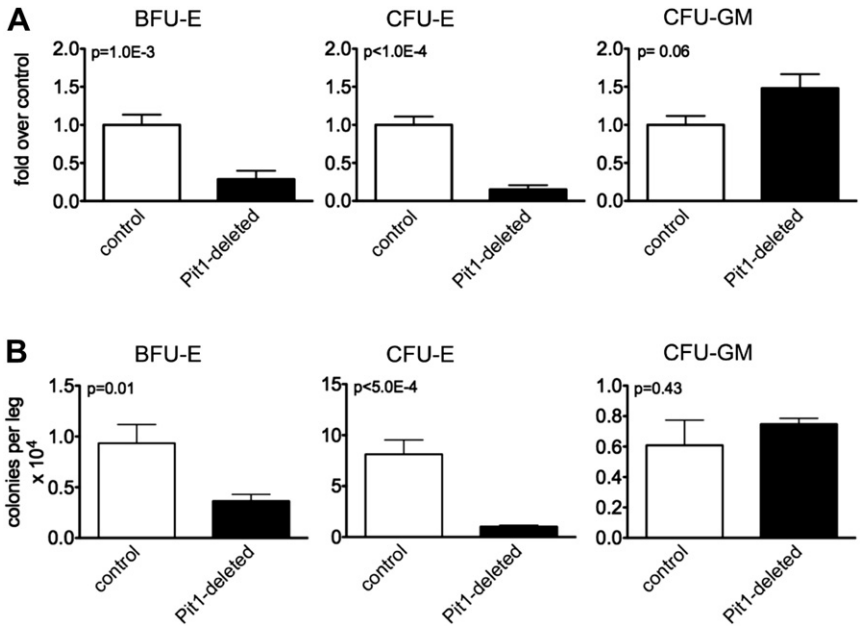
Supplementary Figure E2. (*continued*). **(C)** Expression of *Pit1* mRNA in bone marrow and spleen. mRNA levels were measured by reverse transcriptase quantitative real-time PCR. Sample PCR reactions were performed in triplicate, normalized to β-actin and GAPDH mRNA levels, and expressed as fold-over control mRNA levels. Control mice, n = 5; *Pit1*-deleted mice, n = 5. All data represent mean ± SEM, two-tailed Student *t* test.



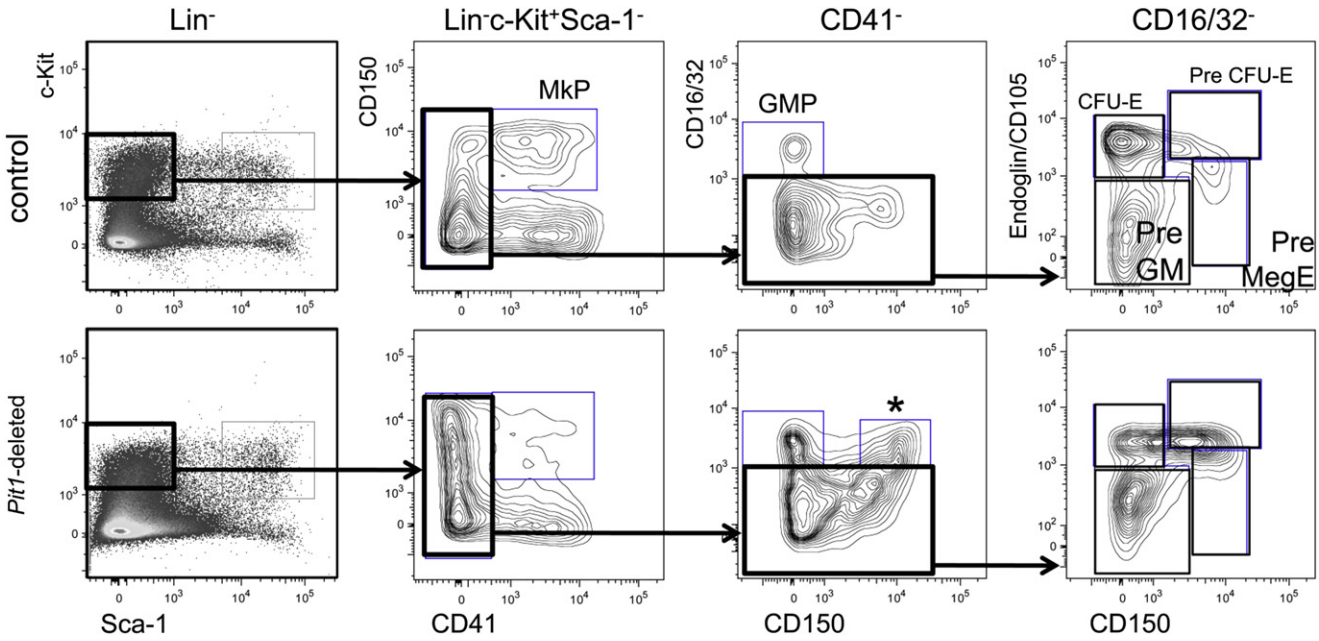
Supplementary Figure E3. *Pit1*-deleted mice have massively enlarged spleens. **(A)** Representative hematoxylin-and-eosin–stained spleen sections of a control and *Pit1*-deleted mouse. The normal spleen architecture is obliterated in *Pit1*-deleted compared to control mice by an expansion of the red pulp space by erythroid precursors. Sections were visualized under a Leica DM1000 upright microscope fitted with a Hi Plan 10×/0.25 objective (Leica, Germany). A Nikon model DS-Fi1 camera and a DS-L2 camera control unit were used to obtain images (Nikon Instruments, Melville, NY, USA). **(B)** The spleen index (spleen weight ÷ body weight) is markedly higher in *Pit1*-deleted mice compared with control mice, calculated as spleen weight/body weight. Spleens were harvested from 4–5-week-old mice, 3–4 weeks after deletion of the floxed allele. Control mice, n = 8; *Pit1*-deleted mice, n = 10; mean ± SEM, two-tailed Student *t* test.



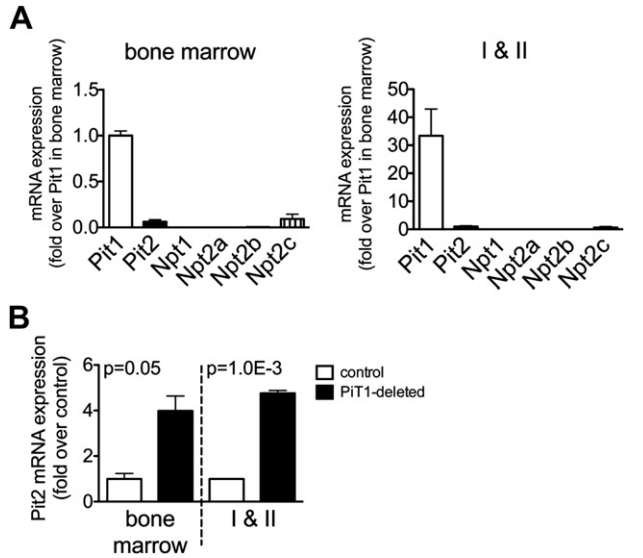
Supplementary Figure E4. Cytospin preparations of whole bone marrow and isolated erythroid cells at different stages of differentiation from *Pit1*-deleted and control mice. Representative Wright-Giemsa-like–stained cytospins of whole bone marrow and the four distinct cell populations shown in Figure 3 (I/II, III, IV, and V). Control sample cells were derived from the pooled whole bone marrow of two control mice. *Pit1*-deleted sample cells were derived from the pooled whole bone marrow of four *Pit1*-deleted mice. Marrow was immunostained and sorted flow cytometrically as described in *Methods*. Cytospins were stained using Hema 3 system (Fisher Scientific) and were visualized under a Leica DM1000 upright microscope fitted with a Hi Plan 100×/1.25 oil objective (Leica, Germany). A Nikon model DS-Fi1 camera and a DS-L2 camera control unit (Nikon Instruments) were used to obtain pictures.



Supplementary Figure E5. Bone marrow–derived CFU-E are reduced in *Pit1*-deleted mice. The comparison of burst-forming units–erythroid (BFU-E), colony-forming units–erythroid (CFU-E), and granulocyte-macrophage colony-forming units (GM-CFU) numbers in deleted mice over control mice are shown. BFU-E colonies from deleted mice were poorly formed compared with colonies from control mice. Colonies counted on day 7 in a BFU-E assay contain erythroid cells that derive from BFU-E present at the time of plating; the assay thus requires that BFU-E differentiate to CFU-E and proerythroblasts and begin hemoglobinization to be enumerated; likewise, the colonies counted on day 2 in a CFU-E assay are a representation of a CFU-E present at the time of plating, and thus require that cells proceed through the proerythroblast stage for inclusion in the CFU-E colony count. Therefore, we expect to see poorly formed BFU-E colonies or fewer BFU-E colony numbers (since they would not meet the criteria of ≥200 cells per colony, which we use with morphology to define BFU-E) and reduced CFU-E colonies when differentiation fails around the proerythroblast stage. Data represent mean ± SEM, two-tailed Student *t* test. **(A)** Data are shown as the fold over control colonies per million cells plated. CFU-E and CFU-GM: control mice, n = 6; *Pit1*-deleted mice, n = 7. BFU-E: control mice, n = 5; *Pit1*-deleted mice, n = 7. **(B)** Data are shown as colonies per mouse leg (femur and tibia). BFU-E, CFU-E, and CFU-GM: control mice, n = 6; *Pit1*-deleted mice, n = 6.



Supplementary Figure E6. Additional characterization of the marrow myeloerythroid progenitor cell compartment in *Pit1*-deleted and control mice. Pre-MegE, Pre CFU-E, CFU-E, MkP, PreGM, and GMP were identified by differential expression of the cell surface markers endoglin (CD105), CD150 (Slamf1), CD41 (Itga2b), CD34, and CD16/32 in $Lin^{-}c\text{-}kit^{+}Sca\text{-}1^{-}$ bone marrow cells. *Pit1*-deleted mice demonstrate a relative expansion of Pre-CFU compared with control mice. We used this method to further characterize the population labeled with the asterisk (in Figure 4B) only observed in *PiT1*-deleted bone marrow. These cells have high cell-surface expression of both endoglin and CD150, suggestive of Pre CFU-E. In addition, we flow the cytometrically sorted population marked by an asterisk, along with CMPs, MEPs, and GMPs and generated cytospin preparations; morphologically, the cells marked by an asterisk resemble MEPs (data not shown). Flow cytometric analyses from a representative control and *Pit1*-deleted mouse, similar results were observed in a total of six deleted and five control mice.



Supplementary Figure E7. Expression of sodium-dependent phosphate import proteins. **(A)** mRNA expression of mammalian sodium-dependent phosphate importers in wild type murine whole bone marrow and sorted proerythroblasts and basophilic erythroblasts. Data expressed as fold-over *Pit1* mRNA expression in bone marrow; n = 2 samples, each containing 2 pooled mice. **(B)** *Pit2* mRNA expression in control and *Pit1*-deleted whole bone marrow and sorted proerythroblasts and basophilic erythroblasts. Data expressed are as fold over control. Control mice, n = 2 with samples containing 2–4 pooled mice; *Pit1*-deleted mice, n = 2with samples containing 2–3 pooled mice. Tissues were harvested from 11-week-old C57BL/6 wild type mice **(A)** or *Pit1*-deleted or control mice **(B)**. For each sample, mRNA levels were measured by reverse transcriptase quantitative real-time PCR. Sample PCR reactions were performed in triplicate and normalized to 18S expression. All data represent mean ± SEM, two-tailed Student *t* test.

Supplementary Table E1. Complete blood counts and reticulocyte counts in PiT1 mutant and control mice[a]

Parameter	Control[b] (n = 7)	*Pit1*-het (n = 7)	*Pit1*-deleted (n = 11)	*p* value
WBC (1,000/μL)	9.72 ± 1.02	8.02 ± 0.81	6.76 ± 0.81	Control vs. Het = 0.22 Control vs. Deleted = 0.04 Het vs. Deleted = 0.31
Neutrophils (1,000/μL)	2.00 ± 0.15	2.07 ± 0.34	1.42 ± 0.18	Control vs. Het = 0.86 Control vs. Deleted = 0.04 Het vs. Deleted = 0.08
Lymphocytes (1,000/μL)	6.79 ± 1.08	5.46 ± 0.53	4.72 ± 0.56	Control vs. Het = 0.29 Control vs. Deleted = 0.08 Het vs. Deleted = 0.38
Hemoglobin (g/dL)	13.01 ± 0.47	12.29 ± 0.39	3.63 ± 0.37	Control vs. Het = 0.25 Control vs. Del ≤ 0.0001 Het vs. Del ≤ 0.0001
MCV (fL)	46.14 ± 0.46	46.71 ± 0.72	50.17 ± 0.89	Control vs. Het = 0.52 Control vs. Deleted = 0.004 Het vs. Deleted = 0.01
Retics × 10^3/μL[c]	523.79 ± 200.15	734.48 ± 207.26	97.67 ± 14.76	Control vs. Het = 0.54 Control vs. Deleted = 0.01 Het vs. Deleted = 0.0024
MCH (pg)	14.61 ± 0.14	14.81 ± 0.22	17.80 ± 0.26	Control vs. Het = 0.45 Control vs. Del ≤ 0.0001 Het vs. Del ≤ 0.0001
Platelets (1,000/μL)	837.30 ± 72.69	899.00 ± 65.54	2082.00 ± 112.90	Control vs. Het = 0.54 Control vs. Del ≤ 0.0001 Het vs. Del ≤ 0.0001

[a]Data obtained 3–4 weeks after deletion of floxed allele (4–5 weeks old). Mean ± SEM, Student *t* test.
[b]Controls include *Pit1*$^{wild\text{-}type/wild\text{-}type}$;*MxCre, Pit1*$^{wild\text{-}type/wild\text{-}type}$, *Pit1*$^{flox/flox}$, *Pit1*$^{flox,wild\text{-}type}$ animals.
[c]For reticulocyte count measurements: control, n = 7; *Pit1*-het, n = 6; and *Pit1*-deleted, n = 13.

Supplementary Table E2. Absolute numbers of hematopoietic progenitors ($\times 10^5$)

Population	Control (n = 2)	*Pit1*-deleted (n = 5)	*p* value
MEP	5.32 ± 0.43	3.79 ± 0.48	0.55
Asterisk (*)	0.07 ± 0.05	0.69 ± 0.18	0.09
GMP	0.82 ± 0.57	0.49 ± 0.09	0.38
CMP	1.29 ± 1.00	0.26 ± 0.07	0.12

CMP = common myeloid progenitor; GMP = granulocyte-macrophage progenitor; MEP = megakaryocytic-erythroid progenitor.

Supplementary Table E3. Serum chemistries in PiT1 mutant and control mice[a]

	Control[b] (n = 11)	*Pit1*-het (n = 7)	*Pit1*-deleted (n = 15)	*p* value
Glucose, mg/dL	218.10 ± 22.19	201.60 ± 42.73	136.5 ± 14.17	Control vs. Het = 0.71 Control vs. Deleted = 0.0028 Het vs. Deleted = 0.07
Calcium, mg/dL	10.05 ± 0.35	9.81 ± 0.25	10.95 ± 0.32	Control vs. Het = 0.64 Control vs. Deleted = 0.07 Het vs. Deleted = 0.04
Phosphorus, mg/dL	12.17 ± 1.11	11.93 ± 0.41	10.58 ± 0.47	Control vs. Het = 0.87 Control vs. Deleted = 0.16 Het vs. Deleted = 0.09
Albumin, g/dL[c]	3.10 ± 0.25	2.64 ± 0.20	3.41 ± 0.20	Control vs. Het = 0.22 Control vs. Deleted = 0.33 Het vs. Deleted = 0.02

[a]Data obtained 3–4 weeks after deletion (4–5 weeks old); mean ± SEM, Student *t* test.
[b]Controls include $Pit1^{wild\text{-}type/wild\text{-}type}$;*MxCre*, $Pit1^{wild\text{-}type/wild\text{-}type}$, $Pit1^{flox/flox}$, $Pit1^{flox,wild\text{-}type}$ animals.
[c]Control, n = 11; *Pit1*-het, n = 7; and *Pit1*-deleted, n = 14.

Supplementary Table E4. Serum chemistries in mice lacking PiT1 in bone marrow and controls[a]

Parameter	Control (n = 4)	*Pit1*-deleted (n = 4)	*p* value
Glucose, mg/dL	157.5 ± 25.6	120.3 ± 39.5	0.16
Calcium, mg/dL	8.7 ± 0.5	9.5 ± 0.6	0.08
Phosphorus, mg/dL	7.3 ± 1.4	7.7 ± 1.2	0.73
Albumin, g/dL	3.0 ± 0.2	3.1 ± 0.6	0.58

[a]Data obtained 7–9 weeks after deletion; mean ± SEM, Student *t* test.