Table S1. Absolute numbers of hematopoietic cells in WT and Ptpn11$^{D61G/+}$ mice

	WT	Ptpn11$^{D61G/+}$
Total cellularity (both hind limbs) [x10^7]	**5.3±0.4**	**5.0±0.4**
LSK [x10^4]	**7.8±0.5**	**15.3±1.1*****
LT-HSC [x10^3]	**5.9±0.9**	**11.5±0.6*****
ST-HSC [x10^3]	**14.2±2.0**	**28.2±5.1***
CMP [x10^4]	**9.18±1.4**	**11.5±2.4**
GMP [x10^4]	**10.5±1.4**	**18.3±3.4***
MEP [x10^4]	**10.9±1.3**	**17.1±2.0***
CLP [x10^4]	**4.7±0.7**	**2.5±0.3****

Absolute numbers of the cells in various hematopoietic compartments in both hind limbs (femurs and tibias) (Mean ± SEM; *, p<0.05; **, p<0.01; ***, p<0.001 by unpaired, two-tailed Student's t test; n=10 per group).

Table S2. Peripheral blood parameters in WT and Ptpn11$^{D61G/+}$ transplants

	WT	Ptpn11$^{D61G/+}$
WBC (K/µl)	**16.1± 2.0**	**29.5±2.0***
Neutrophils (K/µl)	**6.9±1.0**	**12.5±0.9 ****
Monocytes (K/µl)	**0.6±0.1**	**1.2±0.2***

Peripheral blood was analyzed by Hemavet 950F. Data are presented as mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* test; *, $p<0.05$; **, $p<0.01$; n=3 and 7 for WT and *Ptpn11*$^{D61G/+}$ groups, respectively.

Table S3. Peripheral blood parameters in the competitive repopulation assay recipients

	WT:BoyJ	Ptpn11$^{D61G/+}$:BoyJ
Neutrophils (K/μl)	**4.8±1.2**	**12.8±1.9****
Monocytes (K/μl)	**0.5±0.1**	**1.1±0.1***
Lymphocytes (K/μl)	**13.0±1.5**	**8.9±0.5***
Platelets (K/μl)	**466.6±110.0**	**742.2±202.0**
Hemoglobin (g/dL)	**11.3±1.0**	**10.6±0.4**

Peripheral blood was analyzed by Hemavet 950F. Data are presented as mean ± SEM. Statistical significance was determined by unpaired, two-tailed Student's *t* test*;* *, $p<0.05$*;* **, $p<0.01$*;* n=5 per group).

Table S4. Peripheral blood parameters in Ptpn11$^{D61G/+}$/Gab2$^{-/-}$ mice

	WT	Gab2$^{-/-}$	Ptpn11$^{D61G/+}$	Ptpn11$^{D61G/+}$Gab2$^{-/-}$
Neutrophils (K/µl)	**3.5±0.3**	**4.4±0.7**	**8.2±0.5**	**5.4±0.7****
Monocytes (K/µl)	**0.4±0.1**	**0.8±0.2**	**1.3±0.1**	**0.93±0.1***
Lymphocytes (K/µl)	**9.0±0.7**	**9.8±1.4**	**6.0±0.9**	**9.5±1.3***
Neutrophils (%)	**31.4±4.8**	**29.6±2.1**	**53.7±2.1**	**32.3±1.4*****
Lymphocytes(%)	**58.0±4.4**	**56.5±3.2**	**34.4±3.0**	**51.0±1.7*****
RBC (M/µl)	**10.4±0.4**	**10.1±0.4**	**10.5±0.7**	**10.4±0.6**
HCT (%)	**48.1±2.8**	**44.0±1.7**	**47.0±2.9**	**46.5±1.5**
Hb (g/dL)	**14.0±0.7**	**14.7±0.5**	**14.2±0.7**	**14.8±0.8**
Platelets (K/µl)	**439±120.0**	**594±115.0**	**582±137.0**	**424±46.0**

Peripheral blood was analyzed by Hemavet 950F. Data are presented as mean ± SEM. Statistical significance between *Ptpn11$^{D61G/+}$/Gab2$^{-/-}$* and *Ptpn11$^{D61G/+}$* mice were determined by unpaired, two-tailed Student's *t* tests; *, $p<0.05$; **, $p<0.01$; ***, $P<0.001$; n=8 per group. Statistical significance among four groups was verified by one-way ANOVA followed by Tukey's post-test.

Figure S1. MPD phenotypes are reproduced in secondary recipients. BM cells harvested from *Ptpn11*$^{D61G/+}$ and WT littermates were directly transplanted into lethally irradiated BoyJ mice as described in Figure 4. Sixteen weeks following the transplantation, BM cells were harvested and pooled from the primary recipients and transplanted into lethally irradiated BoyJ mice. Peripheral WBC (A and B), spleen weight (C), and percentages of donor-derived myeloid (Mac-1^{+}Gr-1^{+}) cells (D) in the BM of the secondary recipient mice were analyzed 18 weeks post secondary transplantation (n=5). (E) BM cells harvested from WT or *Ptpn11*$^{D61G/+}$ littermates were transplanted with the same number of BoyJ BM cells into lethally irradiated BoyJ mice. Sixteen weeks following the transplantation, BM cells were harvested and pooled from the primary recipients and transplanted into lethally irradiated BoyJ mice. WBC (E) and spleen (F) of the secondary recipient mice were examined 16 weeks post the secondary transplantation (n=5 per group).

Figure S2. Cytokine hypersensitivity of *Ptpn11*$^{D61G/+}$ mutant LSK cells. (A) LSK cells (5x10^{3}) sorted from 4-6 months old WT and *Ptpn11*$^{D61G/+}$ mice were cultured in IL-3 (0 or 2 ng/ml) and 10% FBS-containing IMDM media for 7 days. Total cell numbers were determined (n=3 per group). (B and C) Percentages of myeloid (Mac-1^{+}/Gr-1^{+}) cells differentiated from the sorted LSK cells in the presence of IL-3 (2 ng/ml) were assessed by flow cytometry (n=8 per group). *P* values for comparisons between *Ptpn11*$^{D61G/+}$ and control cells were determined by Student's *t* tests.

Figure S3. Enhanced GM-CSF-induced Erk activation in *Ptpn11*$^{D61G/+}$ macrophages. BM-derived macrophages were generated as described in Materials and Methods. The macrophages were starved in serum and cytokine-free medium for 5 hours and then stimulated with GM-CSF (10 ng/ml) for the indicated periods of time. Whole cell lysates were prepared and examined for Erk activation by immunoblotting with anti-phospho-Erk Ab. Blots were stripped and reprobed with anti-Erk to check protein loading.

Figure S4. Absolute numbers of LSK cells are largely restored in the BM of *Ptpn11*$^{D61G/+}$*/Gab2*$^{-/-}$ mice. WT, *Gab2*$^{-/-}$, *Ptpn11*$^{D61G/+}$, and *Ptpn11*$^{D61G/+}$*/Gab2*$^{-/-}$ mice were generated as described in Figure 6. The mice (n=6 per group) were analyzed for absolute numbers of LSK cells in BM as described in Figure 1. *P* values for comparisons between *Ptpn11*$^{D61G/+}$*/Gab2*$^{-/-}$ and *Ptpn11*$^{D61G/+}$ mice were determined by Student's *t* tests.

Figure S5. Gab1 and Gab3 levels are not significantly altered in *Gab2*$^{-/-}$ or *Ptpn11*$^{D61G/+}$*/Gab2*$^{-/-}$ LSK cells. LSK cells were sorted from WT, *Gab2*$^{-/-}$, *Ptpn11*$^{D61G/+}$, and *Ptpn11*$^{D61G/+}$*/Gab2*$^{-/-}$ mice. RNA was extracted and real-time PCR was performed to determine mRNA levels of Gab1, Gab2, and Gab3.

Figure S6. An important role of Gab2 in mediating the excessive myeloid expansion induced by *Ptpn11*^{D61G} mutation. (A) Mouse Gab2 shRNA lentiviral plasmids were purchased from Open Biosystems (Huntsville, AL). Lentiviral vectors expressing Gab2 shRNAs were generated and used to infect mouse embryonic fibroblasts. Forty-eight hours after infection, RNA was extracted and real-time PCR was performed to determine Gab2 mRNA levels in the

infected cells. (B) BM cells harvested from 4-6 months old *Ptpn11*$^{D61G/+}$ mice were transduced with lentivirus expressing Gab2 shRNA-1 or control lentivirus. Transduced cells (GFP positive) were sorted by flow cytometry and assayed for CFU-GM ($2x10^4$/ml) in the absence or presence of GM-CSF (0.1 ng/ml) or IL-3 (1 ng/ml) as described in Figure 6D, E. (C) Representative CFU-GM colonies from Gab2 shRNA-1 or control virus infected cells.

Figure S1

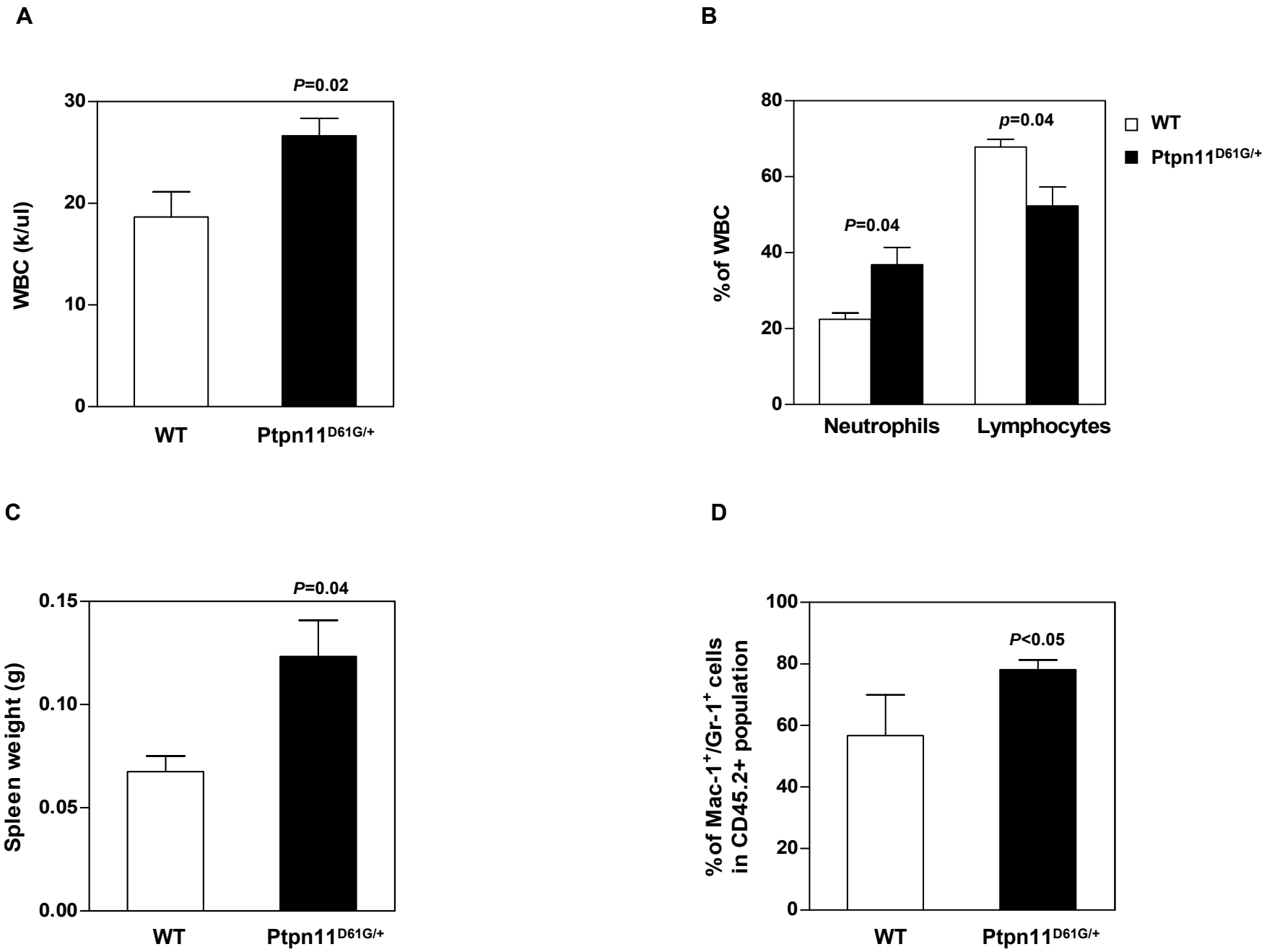
A
P=0.02
WBC (k/ul)
30
20
10
0
WT
Ptpn11D61G/+
B
p=0.04
P=0.04
% of WBC
80
60
40
20
0
Neutrophils
Lymphocytes
WT
Ptpn11D61G/+
C
P=0.04
Spleen weight (g)
0.15
0.10
0.05
0.00
WT
Ptpn11D61G/+
D
P<0.05
% of Mac-1+/Gr-1+ cells in CD45.2+ population
100
80
60
40
20
0
WT
Ptpn11D61G/+

Figure S1

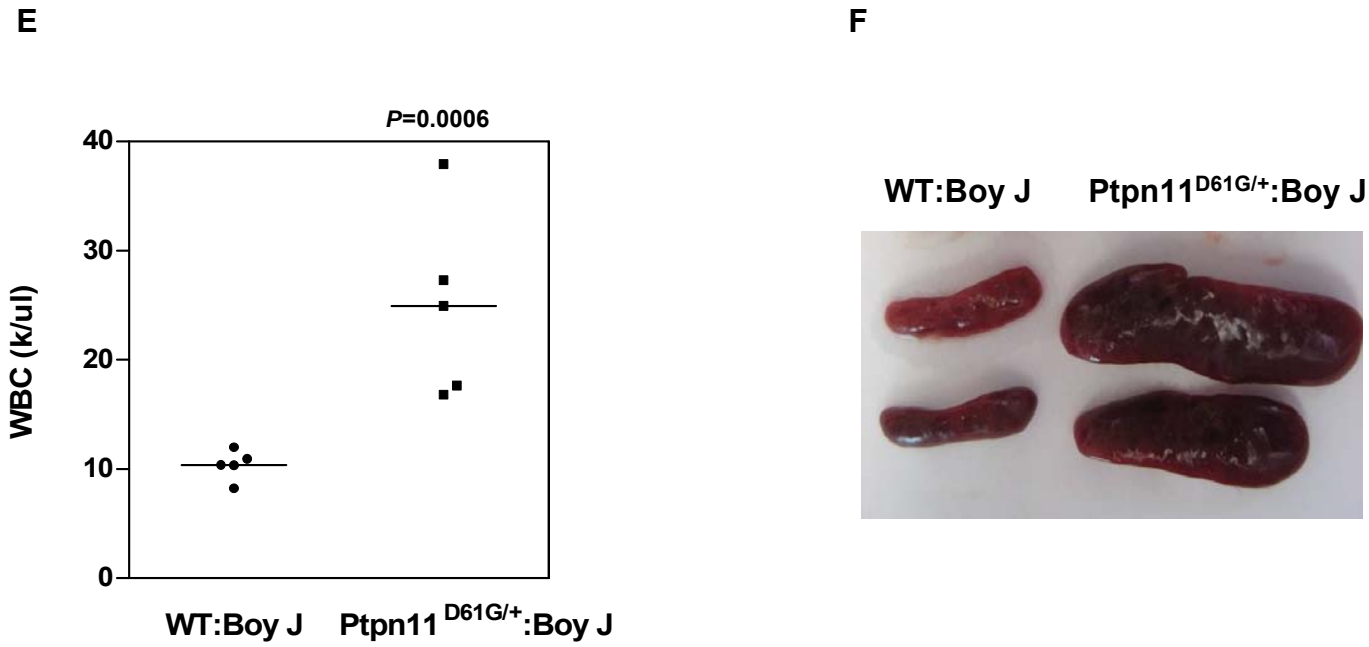
E
P=0.0006
40
30
20
10
0
WBC (k/ul)
WT:Boy J
Ptpn11 D61G/+:Boy J
F
WT:Boy J
Ptpn11D61G/+:Boy J

Figure S2

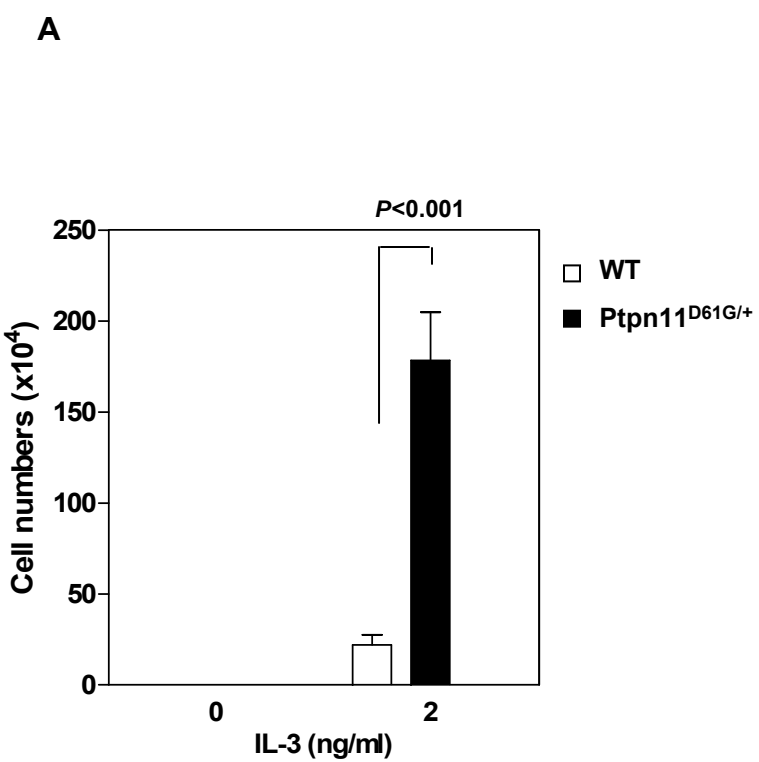
A
P<0.001
250
200
150
100
50
0
Cell numbers (x10^4)
0
2
IL-3 (ng/ml)
WT
Ptpn11D61G/+

Figure S2

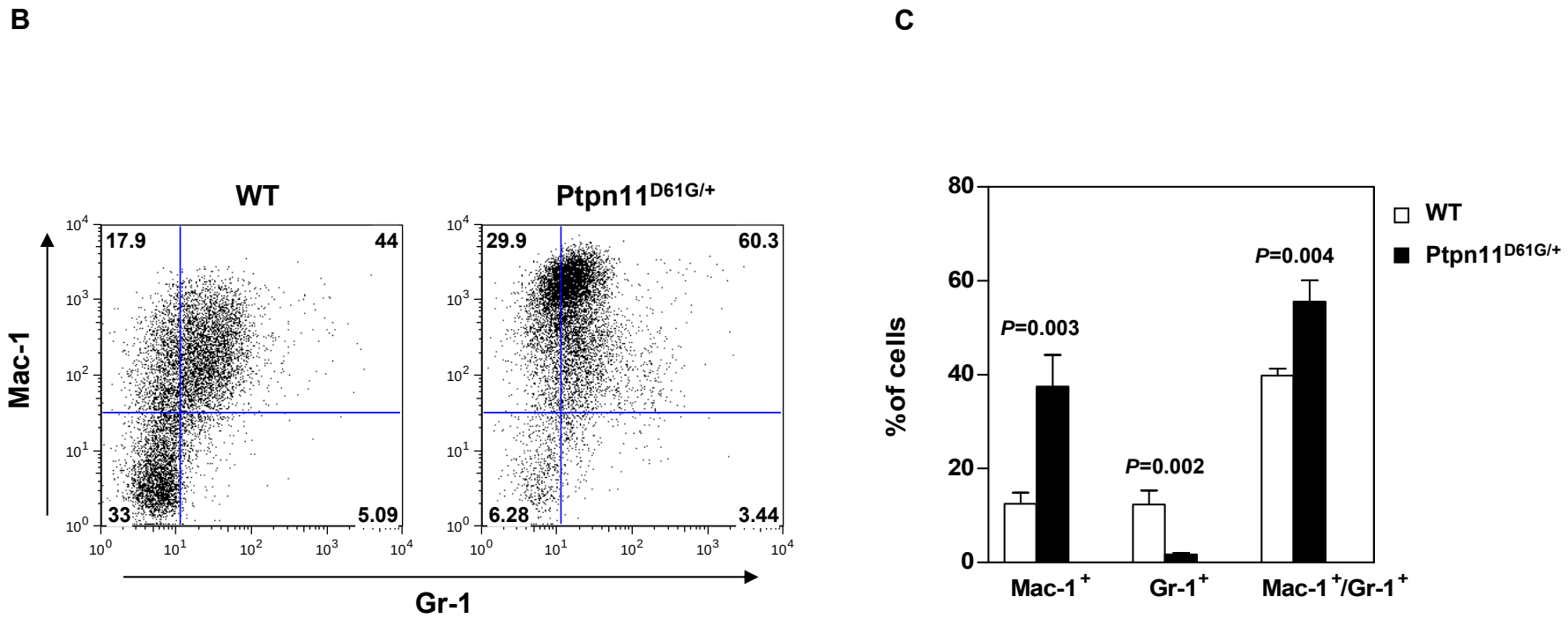
B
WT
Ptpn11D61G/+
Mac-1
Gr-1
17.9
44
33
5.09
29.9
60.3
6.28
3.44
C
%of cells
WT
Ptpn11D61G/+
P=0.003
P=0.002
P=0.004
Mac-1+
Gr-1+
Mac-1+/Gr-1+

Figure S3

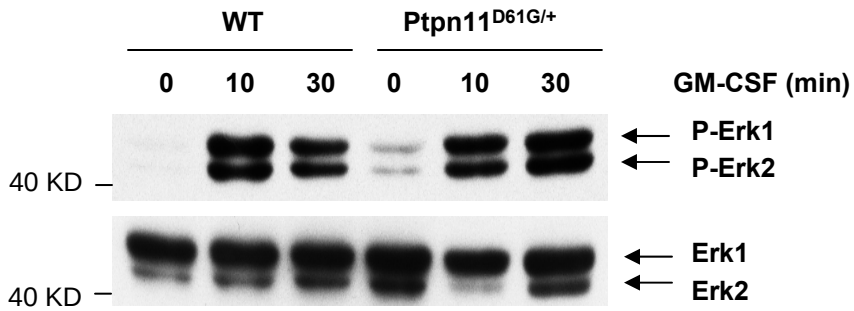
WT
Ptpn11D61G/+
0
10
30
0
10
30
GM-CSF (min)
P-Erk1
P-Erk2
40 KD
Erk1
Erk2
40 KD

Figure S4

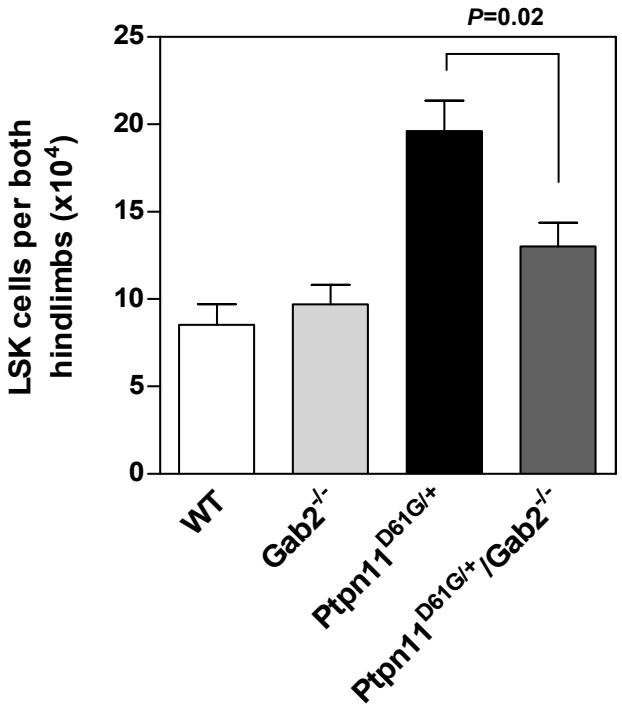
P=0.02
LSK cells per both hindlimbs (x10^4)
25
20
15
10
5
0
WT
Gab2-/-
Ptpn11D61G/+
Ptpn11D61G/+/Gab2-/-

Figure S5

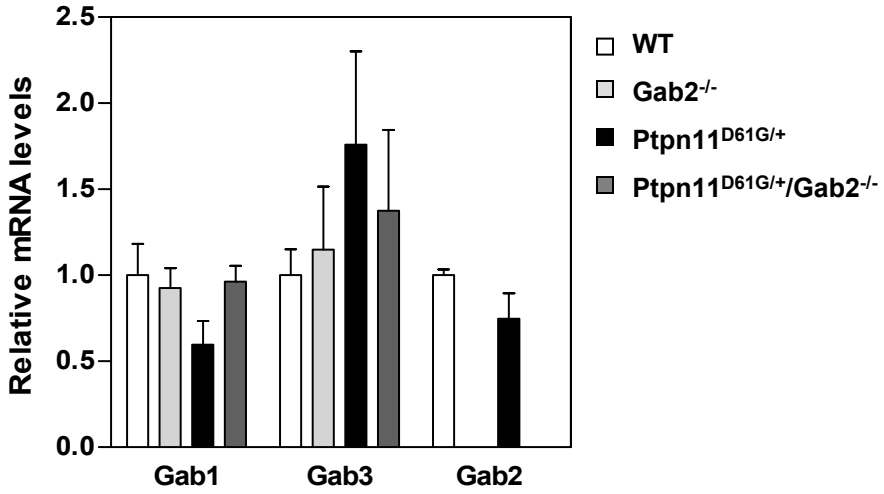
2.5
2.0
1.5
1.0
0.5
0.0
Relative mRNA levels
Gab1
Gab3
Gab2
WT
Gab2-/-
Ptpn11D61G/+
Ptpn11D61G/+/Gab2-/-

Figure S6

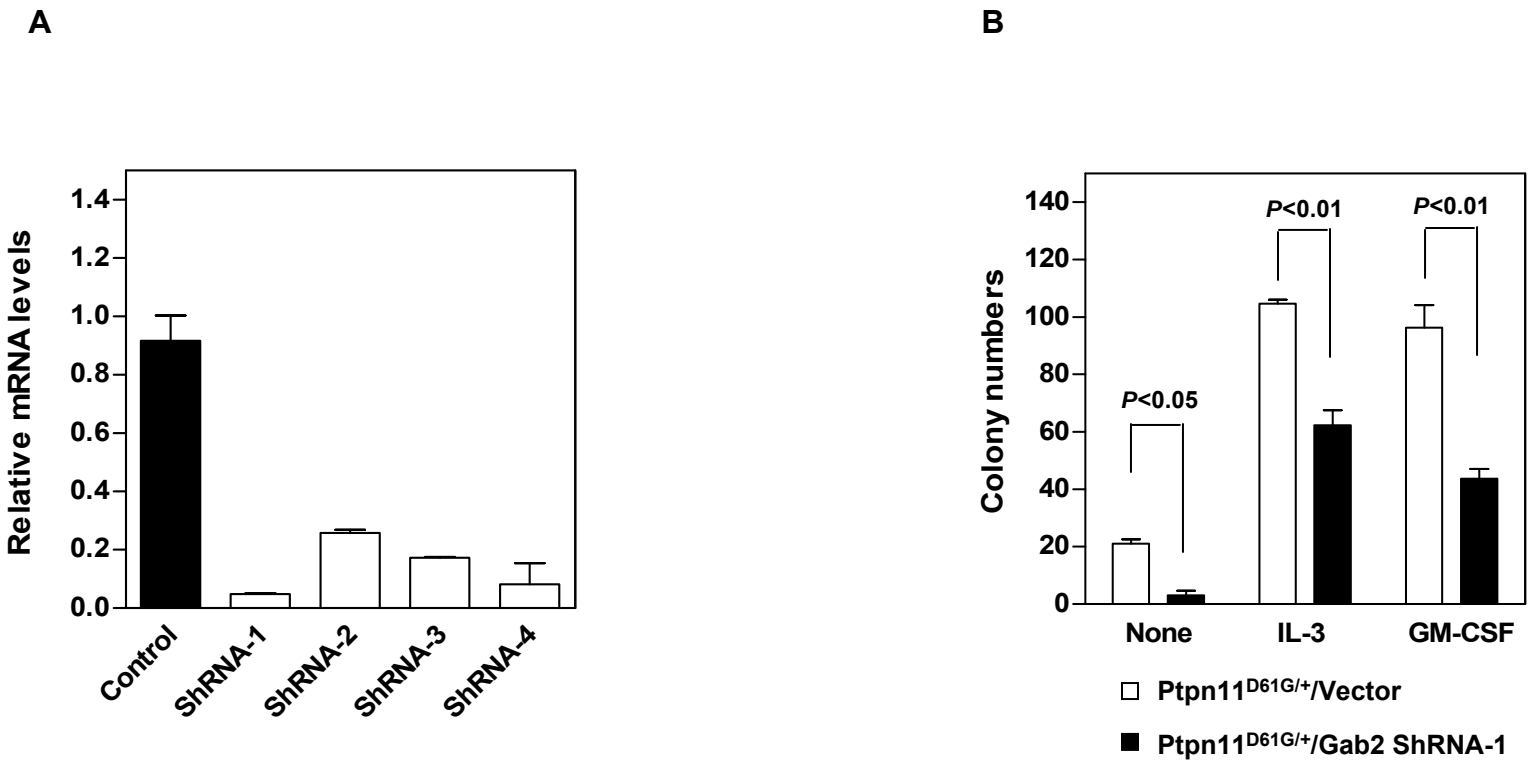
A
Relative mRNA levels
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
ShRNA-1
ShRNA-2
ShRNA-3
ShRNA-4
B
Colony numbers
140
120
100
80
60
40
20
0
P<0.05
P<0.01
P<0.01
None
IL-3
GM-CSF
Ptpn11D61G/+/Vector
Ptpn11D61G/+/Gab2 ShRNA-1

Figure S6

C