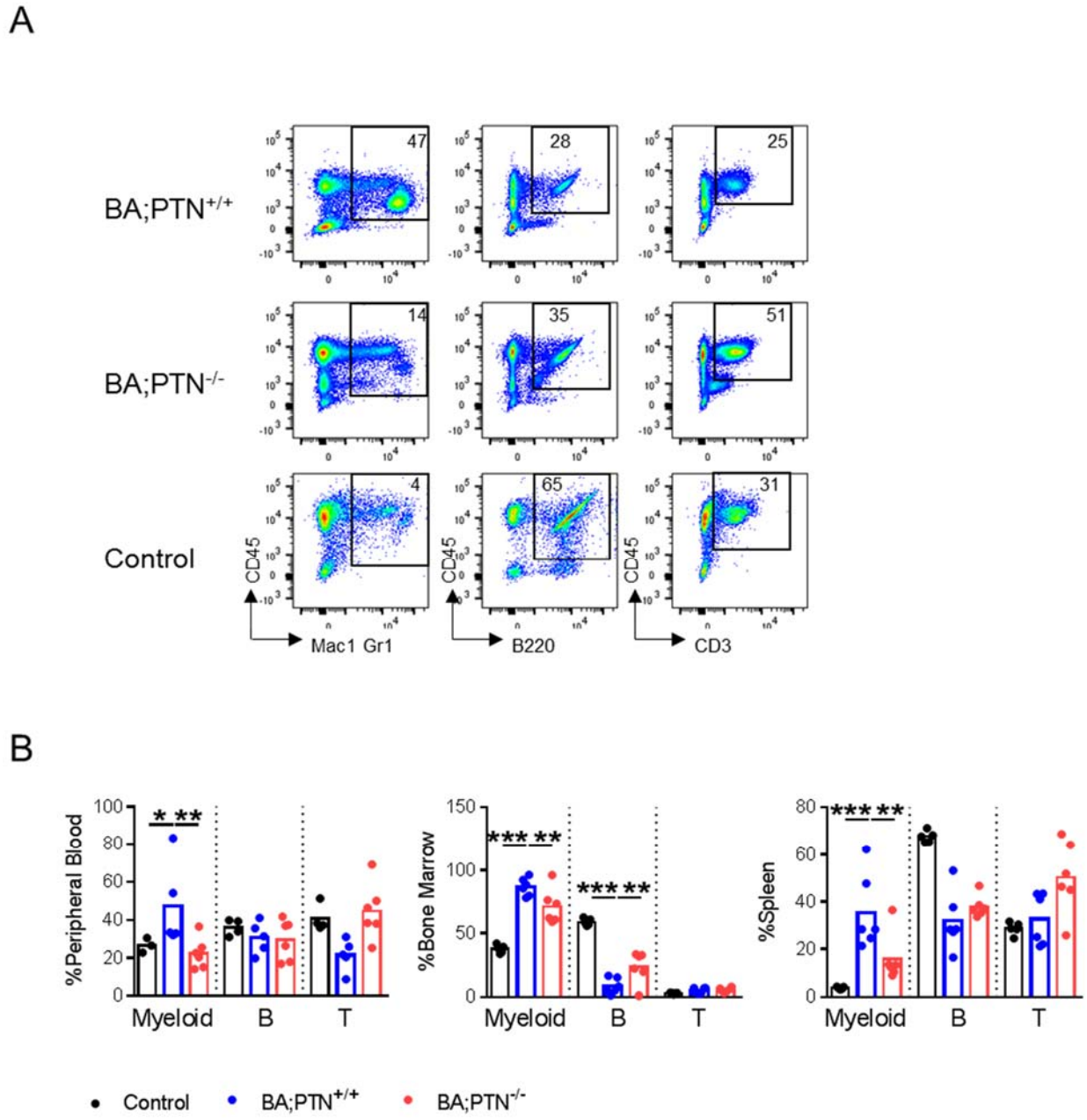
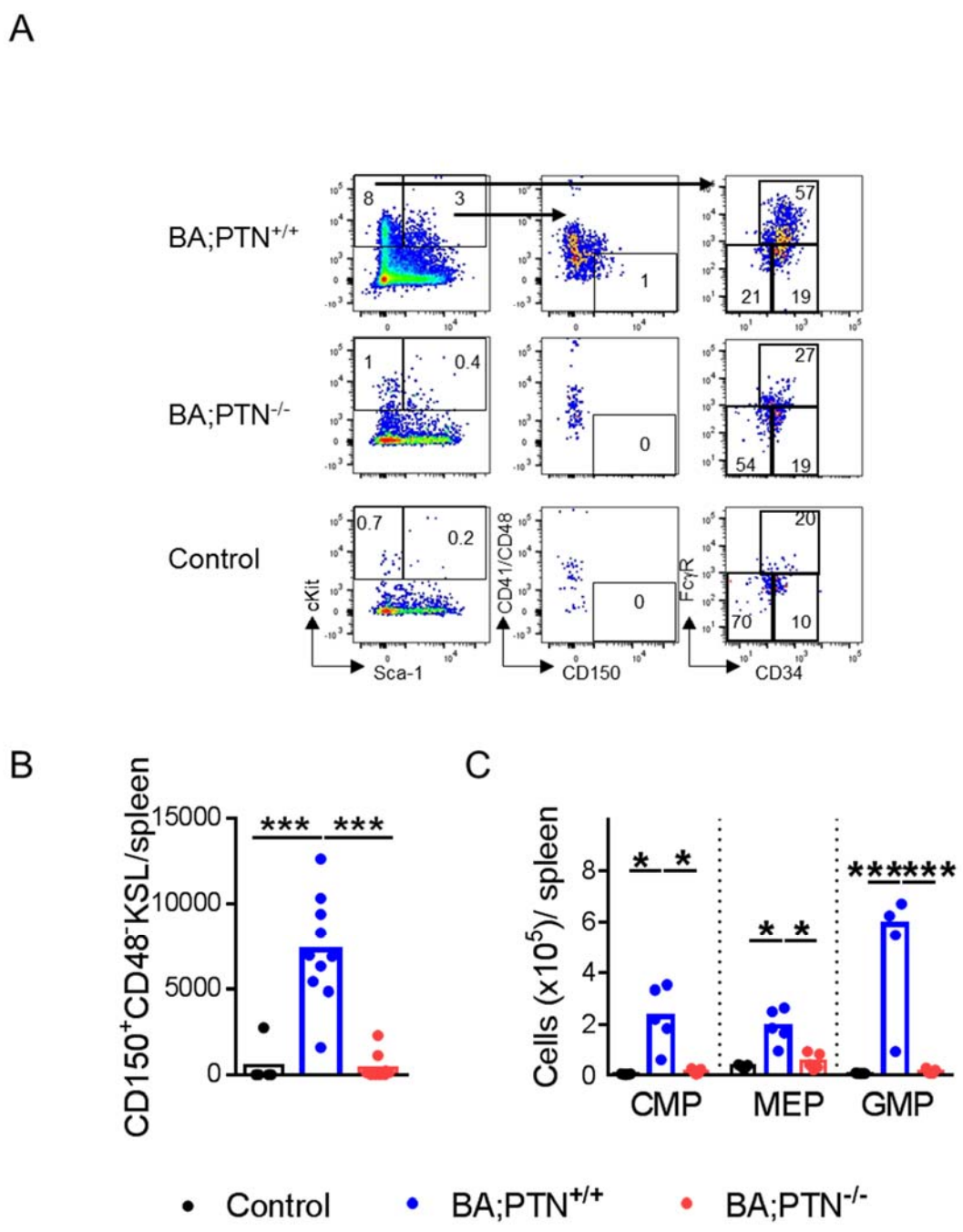
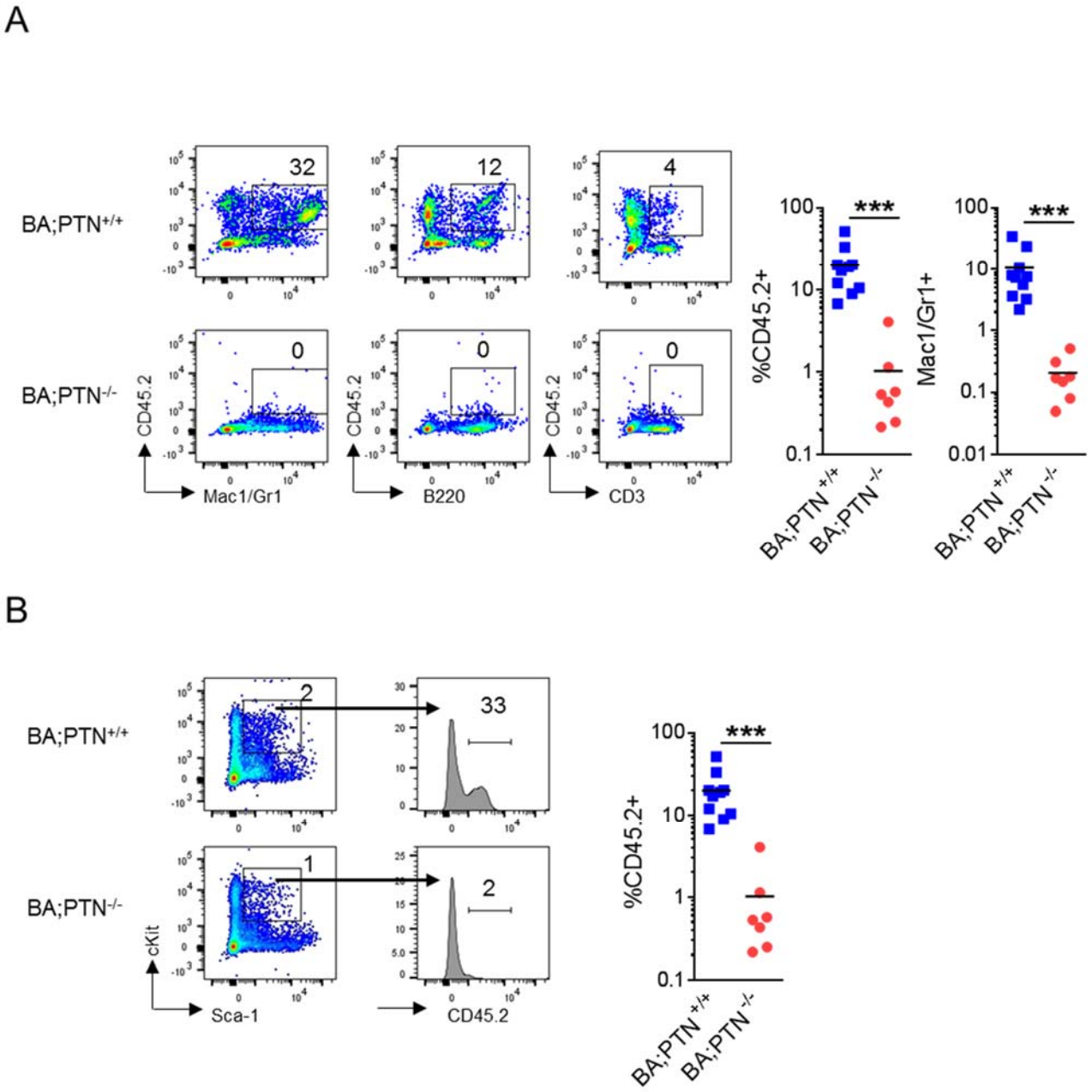
Supplemental Data



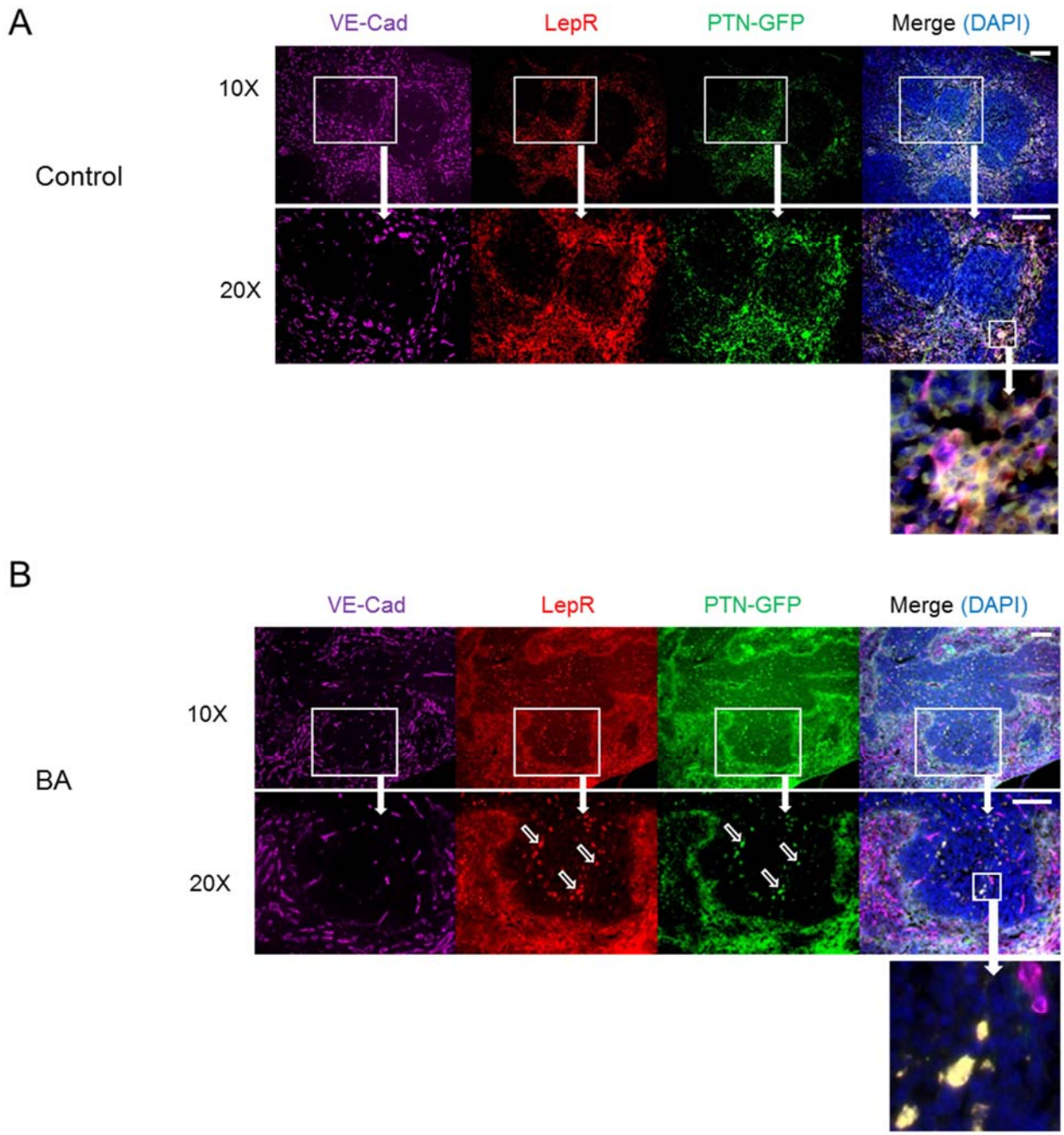
Supplemental Figure 1. PTN deletion decreases myeloid skewing in BA mice.
(**A**) Representative flow cytometric analyses of $Mac1^{+}/Gr1^{+}$ myeloid cells, $B220^{+}$ B cells, and $CD3^{+}$ T cells in the spleens of $BA;PTN^{+/+}$ mice, $BA;PTN^{-/-}$ mice and C57BL/6 mice (controls) at 12 weeks post – BCR/ABL induction. (**B**) Percentages of myeloid cells, B cells and T cells at 12 weeks post – BCR/ABL induction in the PB, BM, and spleens of the mice groups shown (*n*=6/group). Tukey's multiple comparison test for two-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



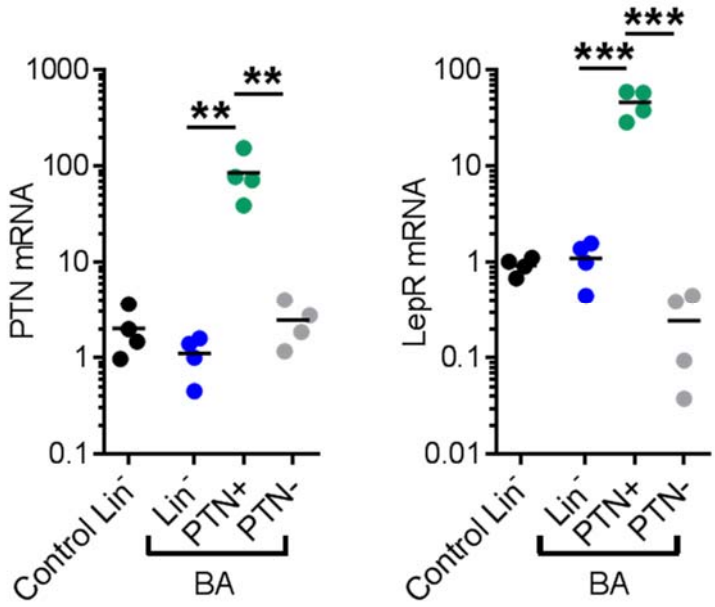
Supplemental Figure 2. PTN deletion decreases CML stem cell enrichment in BA mice. (**A**) Representative flow cytometric analyses of KSL cells, $CD150^{+}CD48^{-}/41^{-}$KSL cells and myeloid progenitor cell populations in the spleens of $BA;PTN^{+/+}$ mice and $BA;PTN^{-/-}$ mice at 12 weeks post – BCR/ABL induction, and controls. $Fc\gamma R^{+}CD34^{+}$ cells are GMPs, $Fc\gamma R^{-}CD34^{-}$ cells are MEPs, and $Fc\gamma R^{-}CD34^{+}$ cells are CMPs. (**B**) Numbers of $CD150^{+}CD48^{-}$KSL cells in the spleens of $BA;PTN^{+/+}$ mice, $BA;PTN^{-/-}$ mice and controls (n=5-10/group). Tukey's multiple comparison test for one-way ANOVA. (**C**) Numbers of MEPs, CMPs, and GMPs in the groups shown at 12 weeks post-BCR/ABL induction (*n*=5/group). Tukey's multiple comparison test for two-way ANOVA. * $P < 0.05$, *** $P < 0.001$.



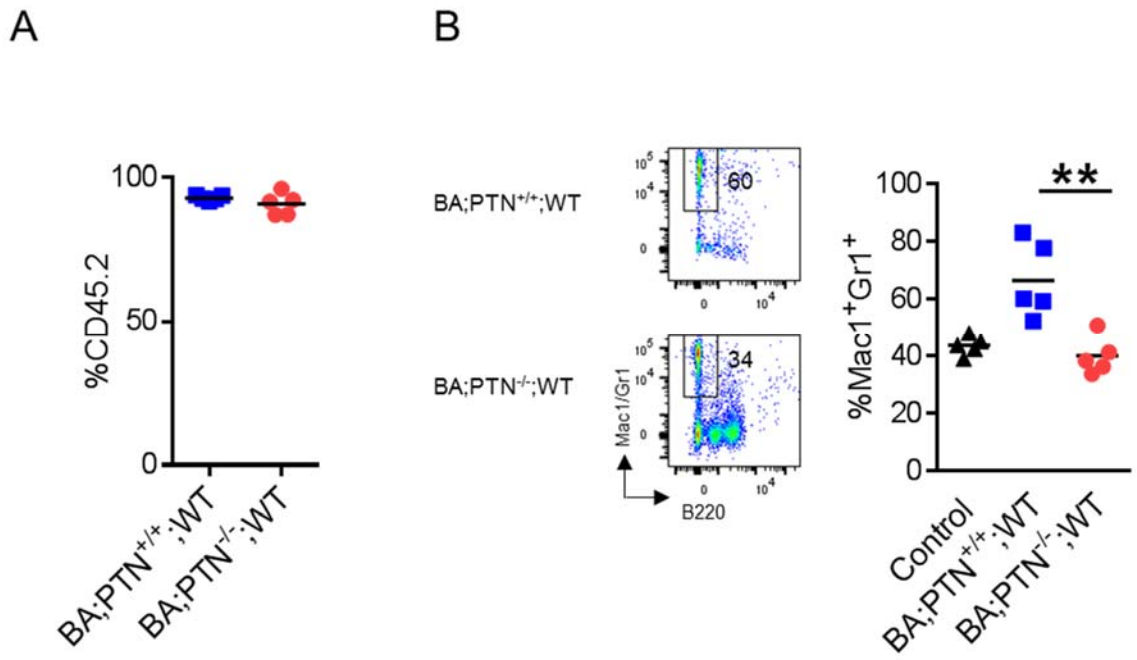
Supplemental Figure 3. PTN deletion decreases CML stem cell repopulating capacity. (**A**) At left, representative flow cytometric analysis of donor CD45.2$^+$ myeloid, B cell, and T cell engraftment in the spleens of recipient (CD45.1$^+$) mice at 10 weeks following transplantation with KSL cells from BA;PTN$^{+/+}$ mice or BA;PTN$^{-/-}$ mice. At right, percentage donor CD45.2$^+$ cells and donor myeloid cell engraftment (*n*=7-9/group); 2-sided Student's t test. (**B**) At left, representative flow cytometric analysis of donor CD45.2$^+$ cell engraftment at 10 weeks within the splenic KSL population in recipient mice transplanted with KSL cells from BA;PTN$^{+/+}$ mice or BA;PTN$^{-/-}$ mice. At right, %CD45.2$^+$KSL cells in the spleen of the recipient mice shown (*n*=7-9 per group); 2-sided Student's t test. $^{**}P < 0.01$, $^{***}P < 0.001$.


A
VE-Cad
LepR
PTN-GFP
Merge (DAPI)
10X
Control
20X
B
VE-Cad
LepR
PTN-GFP
Merge (DAPI)
10X
BA
20X
C
Control
CD45
7AAD
70
30
CD45+
PTN
LepR
0
BA
CD45
7AAD
94
6
CD45+
PTN
LepR
1.1
%PTN+LepR+
1.5
1.0
0.5
0.0
***
Control
BA

Supplemental Figure 4. **A subset of CD45$^+$ cells express PTN and LepR in BA mice**. (**A**) Cross sections of spleens of PTN-GFP control mice are shown. Magnification of each image shown at left. In control mice, PTN expression (green) co-localized with VEcad$^+$ ECs (magenta) and LepR$^+$ stromal cells (red) in the red pulp region. Nuclei were counterstained with DAPI. The inset box shows a further magnified, merged image revealing the co-localization of PTN expression with LepR$^+$ cells and VEcad$^+$ ECs (yellow). (**B**) Cross sections of BA;PTN-GFP mice (BA) at 12 weeks following *BCR/ABL* induction. Corruption of the normal splenic architecture is demonstrated. In the highlighted region (inset box, 10x), PTN expression co-localized with LepR$^+$ cells (white arrows, 20x images) and was distinct from VEcad$^+$ vascular structures. The magnified, merged image displays PTN-expressing cells co-localized with LepR expression (yellow). (**C**) Representative flow cytometric analysis of expression of PTN and LepR in the splenic CD45$^+$ population of control mice and BA mice at 12 weeks following *BCR/ABL* induction. At right, percentages of PTN$^+$LepR$^+$ cells within the CD45$^+$ population are shown for each group (n=3 mice/ group); 2-sided Student's t test. ***$P < 0.001$.



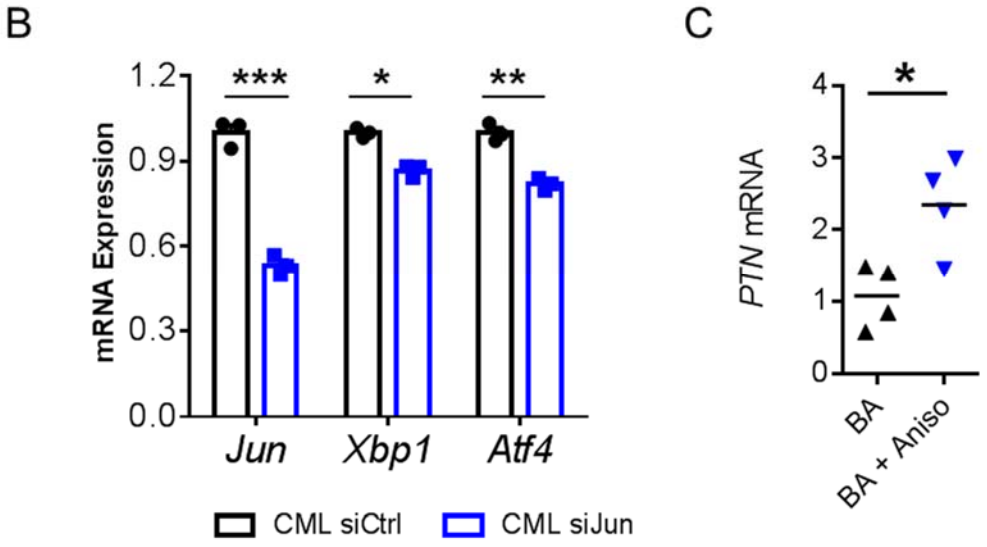
Supplemental Figure 5. PTN^{+} cells in the spleen of BA mice are enriched for *LepR* expression. At left, *Ptn* gene expression is shown in the cell populations shown from the spleens of PTN-GFP (control) mice and from BA;PTN-GFP mice (BA) at 12 weeks post-*BCR/ABL* induction. At right, *LepR* gene expression is shown in the same splenic cell populations (*n*=4 mice/group); Dunnett's multiple comparison test for one-way ANOVA. ** $P < 0.01$, *** $P < 0.001$.



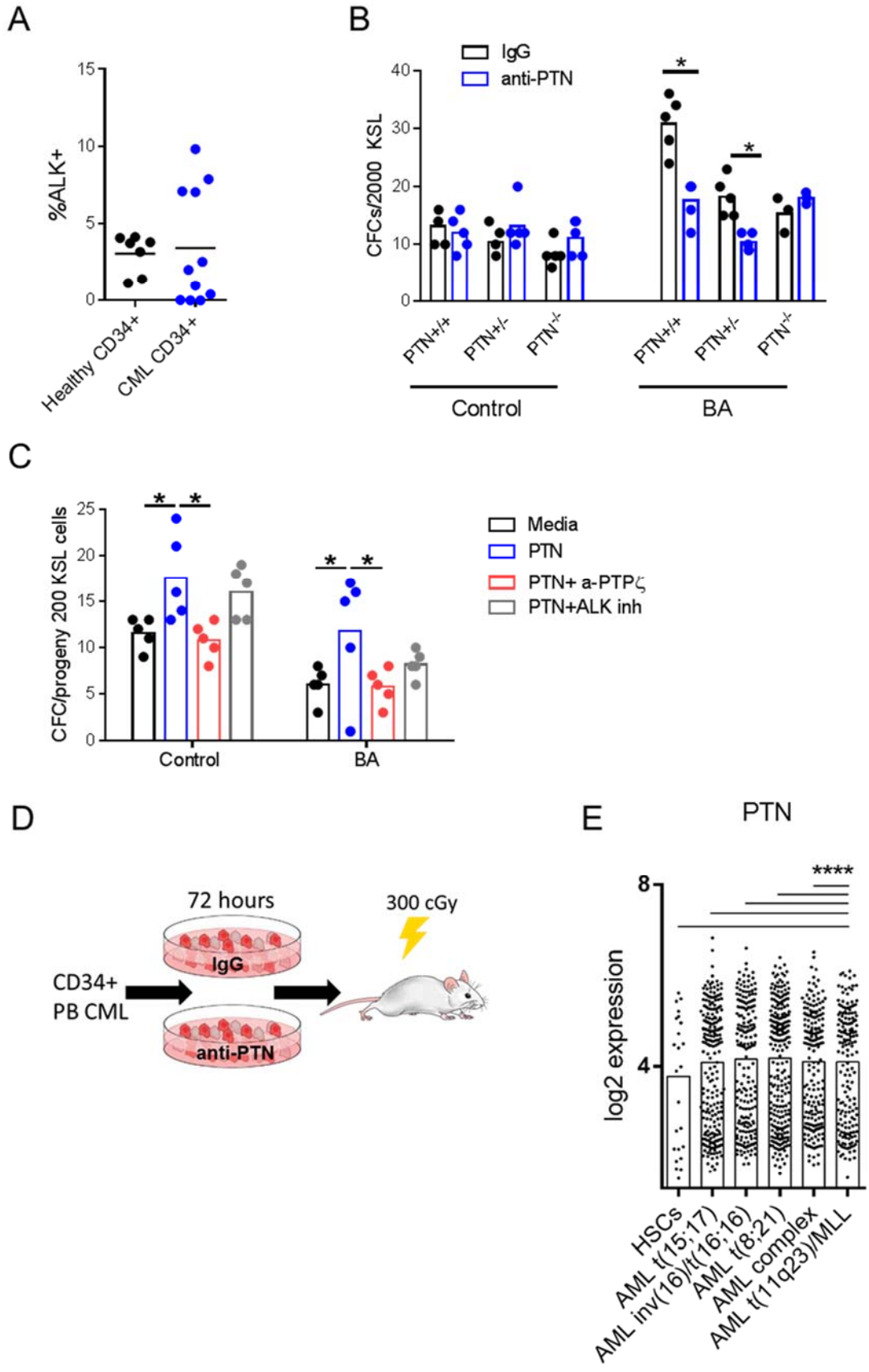
Supplemental Figure 6. Hematopoietic cell – specific deletion of PTN abrogates CML pathogenesis in vivo. (**A**) Donor hematopoietic cell engraftment at 4 weeks post-transplant in chimeric mouse model (n=5/group). (**B**) At left, representative flow cytometric analysis of $Mac1^+/Gr1^+$ myeloid cells in the BM of wild type (WT) mice transplanted with BM cells from $BA;PTN^{+/+}$ mice or $BA;PTN^{-/-}$ mice, at 16 weeks post-BCR/ABL induction. At right, percentages of $Mac1^+/Gr1^+$ cells in the BM of $BA;PTN^{+/+}$;WT mice and $BA;PTN^{-/-}$;WT mice are shown, compared to adult B6.SJL mice controls (*n*=5/group). Student's t test. ** $P < 0.01$.

A

Gene Symbol	Fold Change (BA;PTN-/- vs BA;PTN+/+)	p-value
Jun	-2.2	0.002
Jak2	-1.58	0.004
Mlh1	-1.59	0.012
Lmo2	-1.92	0.014
Il12a	-2.98	0.023
Cdc42ep3	-1.54	0.025
Cdkn1a	-1.85	0.033
Grb2	-1.32	0.040
Akt1	-1.73	0.046



Supplemental Figure 7. PTN induces *Jun* and *UPR* gene expression. (**A**) The table shows the genes downregulated by at least 1.5-fold in KSL cells from BA;PTN$^{-/-}$ mice versus BA;PTN$^{+/+}$ mice ($P < 0.05$). *Jak2* = janus kinase 2, *Mlh1* = MutL homologue 1, *Lmo2* = LIM domain only 2, *IL12a* = interleukin-12 alpha, *CDC42ep3* = Cdc42 effector protein 3, *Cdkn1a* = cyclin-dependent kinase inhibitor 1a; *Grb2* = growth factor receptor bound protein 2, *Akt1* = serine/threonine protein kinase 1. (**B**) Expression of *Jun*, *Atf4* and *Xbp1* in splenic KSL cells from BA mice at 72 hours following treatment with *Jun* siRNA or sham siRNA (*n*=3 mice/group), Sidak's multiple comparison test for two-way ANOVA. (**C**) PTN mRNA levels in KSL cells from BA mice at 2 hours following anisomycin – induction of *Jun* expression (n=4/group). Student's t test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.


A
%ALK+
15
10
5
0
Healthy CD34+
CML CD34+
B
IgG
anti-PTN
CFCs/2000 KSL
40
30
20
10
0
*
*
PTN+/+
PTN+/-
PTN-/-
PTN+/+
PTN+/-
PTN-/-
Control
BA
C
CFC/progeny 200 KSL cells
25
20
15
10
5
0
*
*
*
*
Control
BA
Media
PTN
PTN+ a-PTPζ
PTN+ALK inh
D
72 hours
300 cGy
CD34+
PB CML
IgG
anti-PTN
E
PTN
****
log2 expression
8
4
HSCs
AML t(15;17)
AML inv(16)/t(16;16)
AML t(8;21)
AML complex
AML t(11q23)/MLL

Supplemental Figure 8. PTN inhibition differentially suppresses CML colony formation. (**A**) Scatter plot of %ALK^+CD34^+ cells from healthy adults and CML patients (*n*=5 healthy, *n*=12 CML). (**B**) Numbers of CFCs per 2 x 10^3 KSL cells from $PTN^{-/-}$, $PTN^{+/-}$ and $PTN^{+/+}$ mice (Control) compared to that from the same dose of KSL cells from BA;$PTN^{-/-}$, BA;$PTN^{+/-}$ and BA;$PTN^{-/-}$ mice, treated with 10 μg/ml anti-PTN or IgG (*n*=3-5/group). (**C**) Numbers of CFCs generated from KSL cells from C57BL/6 mice (Control) or BA mice treated with 200 ng/ml PTN with or without 5 ug/ml anti-PTPζ (a-PTPζ) or 3 nM TAE684 (ALK inh)(*n*=5/group). (**D**) Human CML xenotransplantation model in NSG mice. (**E**) PTN gene expression in human HSPCs (*n*= 24), AML t(15;17)(*n*=217), AMLinv(16)(*n*=189), AML (8;21)(n=241), AML complex (*n*=192), AML t(11q23)/MLL (*n*=172). Sidak's multiple comparison test for two-way ANOVA for (**B**) and Dunnett's multiple comparison test for two-way ANOVA for (**C**). *$P < 0.05$, **** $P < 0.0001$ for normal HSPCs versus each AML subtype.

Supplemental Table 1. Flow cytometry antibodies

Antibody	SOURCE	Catalog Number
Endothelial and Leptin Receptor Cell Identification		
Anti-mouse VE-Cadherin AF647	Biolegend	138006
Anti-LEPR / Leptin Receptor Antibody PE	LifeSpan Biosciences	LS-C261834
7-AAD Staining Solution	BD Biosciences	559925
Donor/Host Hematopoietic Cell Discrimination		
CD45.1 PE Mouse anti-Mouse	BD Biosciences	553776
CD45.2 FITC Mouse anti-Mouse	BD Biosciences	553772
Mature Hematopoietic Lineages Analysis		
B220 (CD45R) APC-Cy7 Rat Anti-Mouse	BD Biosciences	552094
Gr-1 (Ly-6G and Ly-6C) PE Rat anti-Mouse	BD Biosciences	553128
Mac-1 (CD11b) PE Rat anti-Mouse	BD Biosciences	557397
CD3 V450 Rat Anti-Mouse Molecular Complex	BD Biosciences	561389
Hematopoietic Stem and Myeloid Progenitor Analysis		
CD41 Alexa Fluor 488 anti-mouse	Biolegend	133908
CD150 Alexa Fluor 647 Rat anti-Mouse	BD Biosciences	562647
c-kit (CD117) PE Rat anti-Mouse	BD Biosciences	553355
Sca-1 (Ly-6A/E) APC-Cy7 Rat anti-Mouse	BD Biosciences	560654
V450 Mouse Lineage Antibody Cocktail	BD Biosciences	561301
CD34 FITC	BD Biosciences	553733
CD16/32 (Fcγ R III/II) APC	Biolegend	101325
CD48 AF488	Biolegend	HM48-1
Human Hematopoietic Cell Analysis		
Human CD45 V450	BD Biosciences	560367
Human CD33 PE	Biolegend	366608
p-PERK Analysis		
Anti-PERK (Phospho T982)	Abcam	ab192591
Alexa Fluor 488 labeling kit	ThermoFisher	A20181