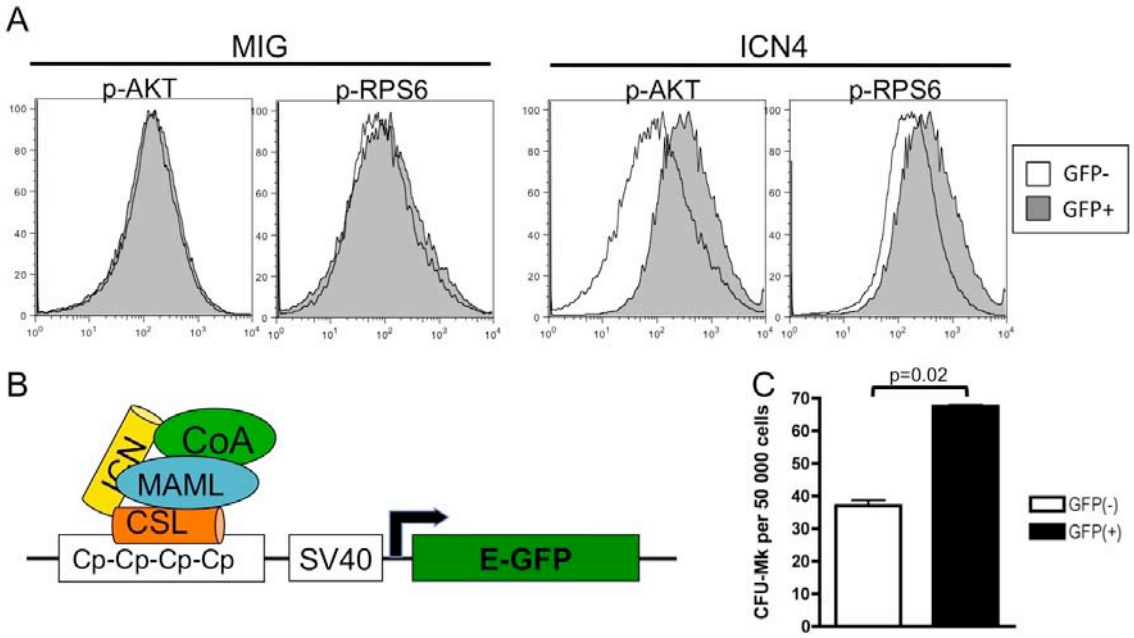

Figure S1. Analysis of TNR and ICN4 transplant mice

(A) Flow-sorted Lineage$^-$, propidium iodide$^-$ (Lin$^-$PI$^-$) cells from either MSCV-IRES-GFP or MIG-ICN4 infected bone marrow recipients were fixed/permeabilized and prepared for phospho-flow analyses as described in Figure 1. Histograms show phosphorylation levels of AKT and RPS6 in the GFP$^+$ vs. the GFP$^-$ compartment within the same animals and are a representative of three independent animals analyzed.

(B) Scheme depicting the TNR mouse model. The transgenic construct consists of four tandem copies of the C-promoter binding factor 1 (CBF1/RBPSUH) binding site of the Epstein-Barr virus major latency C promoter (Cp), followed by the SV40 minimal promoter and the coding sequence for EGFP. Expression of EGFP happens when the promoter is activated, by binding of a complex comprising RBPJ, MAML1 and ICN to the upstream RBPJ binding elements.

(C) MegaCult assay with GFP$^+$ and GFP$^-$ whole bone marrow cells for assessment of CFU-MK potential. Mean±SEM of triplicate experiments is represented.

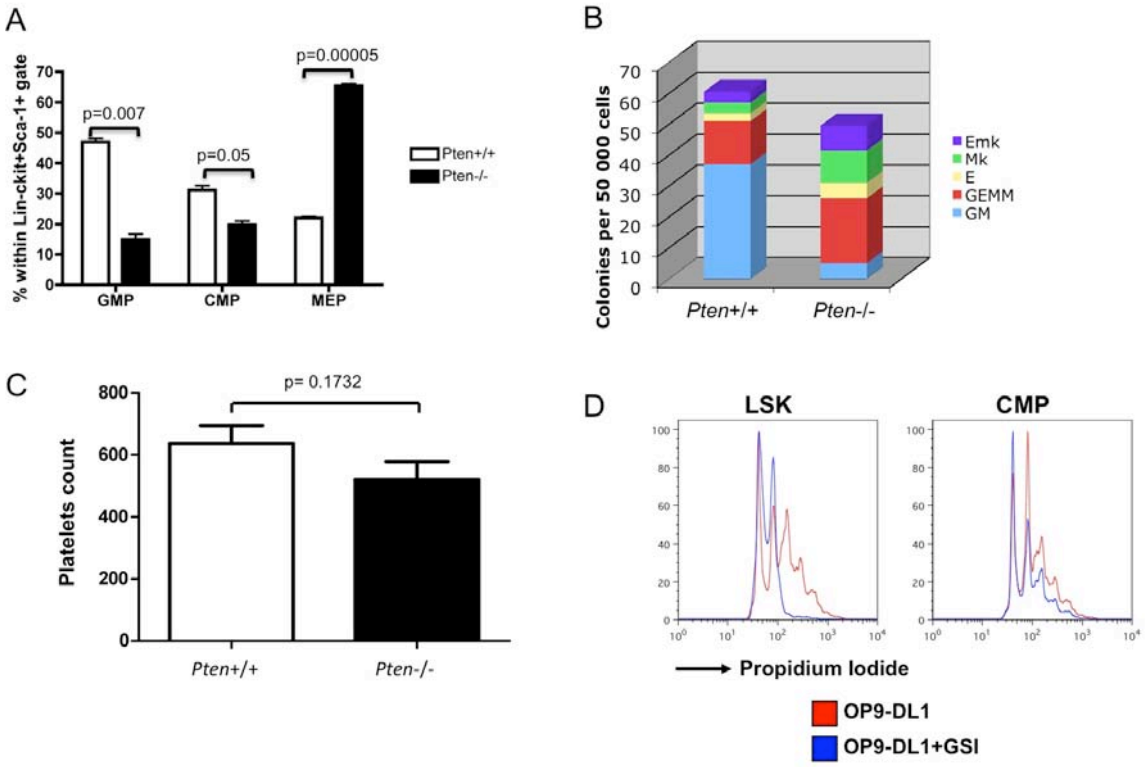

Figure S2. Analysis of *Pten*-deficient animals

(A) Flow cytometric analysis of myeloid progenitors within the Lineage^{-}cKit^{+}Sca1^{-} population of *Pten*$^{+/+}$ and *Pten*$^{-/-}$ mice two weeks post pIpC injection. Bar graphs show the mean±SEM of five independent experiments.

(B) Whole bone marrow cells from *Pten*$^{+/+}$ and *Pten*$^{-/-}$ mice were plated in M3434 methylcellulose media supplemented TPO. Colonies were scored after seven days. GM: Granulocyte-Macrophage colony, GEMM: Granulocyte-Macrophage-Erythroid-Megakaryocyte colony, EMk: Erythroid-Megakaryocyte colony, E: Erythroid colony, Mk: Megakaryocyte colony.

(C) Bar graphs representation of the platelet counts showing the mean±SEM platelets count for *Pten*$^{+/+}$ (n=12) and *Pten*$^{-/-}$ (n=11) mice.

(D) Ploidy analysis was performed on hematopoietic cells obtained from PTEN $^{-/-}$ LSK and CMP plated on OP9-DL1+/-GSI for 6 and 4 days, respectively. Analysis was gated on CD45+CD41+ cells as shown in Figure 3F, 3G.

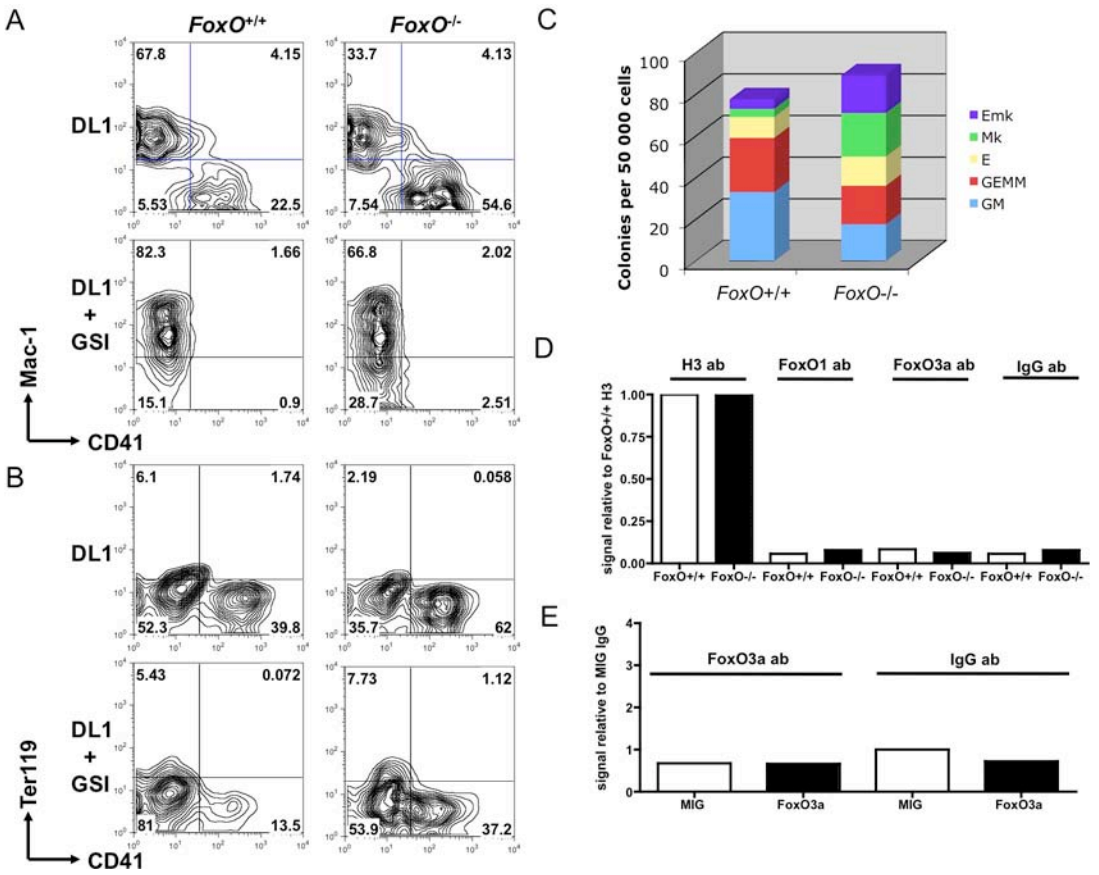

Figure S3. Megakaryocytic potential of *FoxO*-deficient cells

(A) LSK cells from *FoxO1/3/4*cKO-Mx1Cre^{-} (*FoxO*$^{+/+}$) or *FoxO1/3/4*cKO-Mx1Cre^{+} (*FoxO*$^{-/-}$) mice were flow-sorted three weeks post pIpC treatment and cultured on OP9-DL1 stroma in the presence or absence of 1μM GSI. After six days of co-cultures, cells were analyzed by flow cytometry for the development of CD41^{+} cells within the CD45^{+} gate.

(B) CMP cells from *FoxO*$^{+/+}$ or *FoxO*$^{-/-}$ mice were purified and analyzed as in (A).

(C) Whole bone marrow cells from *FoxO*$^{+/+}$ or *FoxO*$^{-/-}$ animals were plated in M3434 methylcellulose media supplemented with 50ng/ml TPO. Colonies were scored after seven days. GM: Granulocyte-Macrophage colony, GEMM: Granulocyte-Macrophage-Erythroid-Megakaryocyte colony, EMk: Erythroid-Megakaryocyte colony, E: Erythroid colony, Mk: Megakaryocyte colony.

(D) Chromatin Immunoprecipitation assay (ChIP) of *FoxO*$^{+/+}$ or *FoxO*$^{-/-}$ Lin^{-} cells using an anti-FOXO1 or an anti-FOXO3a antibody to test binding of these factors to the 3' untranslated region (UTR) region of the Hes1 gene. Bar graphs represent the mean±SEM of triplicate experiments and all signals are normalized to FoxO$^{+/+}$ sample pulled down with anti-Histone H3 antibody

(E) ChIP analysis of Lin^{-} cells infected with an empty MIG vector or with a wildtype FoxO3a construct and pulled down with either an anti-FOXO3a antibody. All signals at the Hes-1 3'UTR region are normalized to MIG cells pulled down with an anti-IgG antibody and bar graphs represent the mean±SEM of triplicate experiments.