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Supplemental Data

Hedgehog Signaling Is Dispensable for Adult Murine Hematopoietic Stem Cell Function and Hematopoiesis

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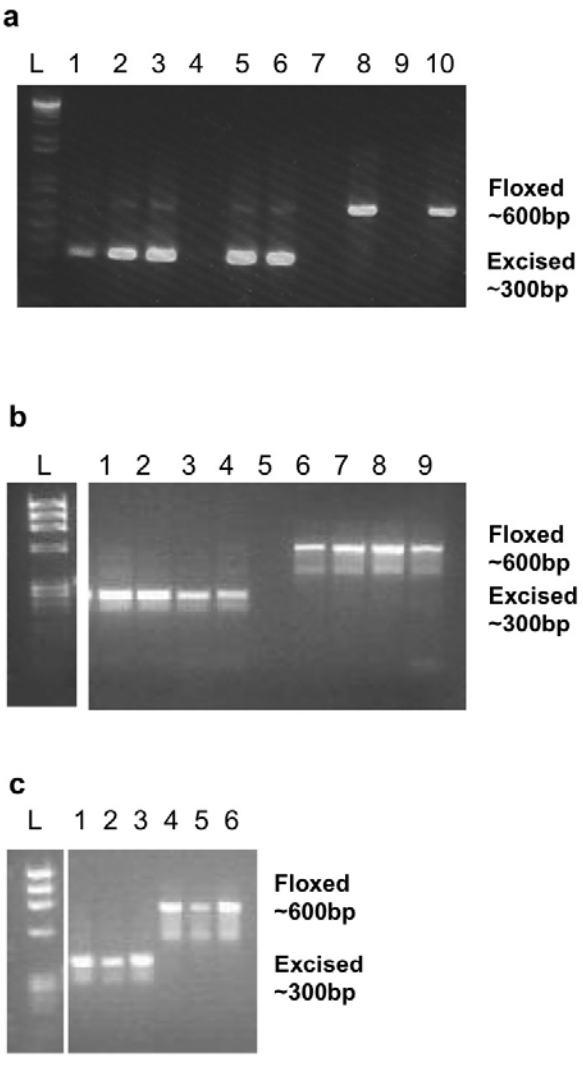

Figure S1. Long-term adult hematopoiesis is sustained by *Smo* deficient hematopoietic cells

(a) *Smo*^{Null} or *Smo*^{WT} mice (n=3-6 per group) were treated with ~25 μg/gram pIpC at 10 weeks of age. Complete *Smo* excision via 3-primer PCR reaction was observed 6 months after pIpC injection in the peripheral blood. L = molecular weight markers, Lanes 1-6 = *Smo*^{Null} blood samples derived from individual animals showing an excised *Smo* allele as a 300bp product; Lane 7=H2O control; Lanes 8-10 = *Smo*^{WT} samples showing the intact floxed *Smo* allele with a 600bp product. Lanes 4 and 9 had suboptimal amplification. **(b)** Comparable mouse experiment (n=10 for *Smo*^{Null} and n=4 for *Smo*^{WT}) as in **(a)**, showing persistence of the excised *Smo* allele in the bone marrow 18 months after i.p. injection of 600 μg (=25 μg/gram) pIpC. L = molecular weight markers, Lanes 1-4 = *Smo*^{Null} samples, Lane 5 = H2O control, Lanes 6-9 = S*mo*^{WT} samples. **(c)** Demonstration of *Smo* excision in a competitive bone marrow transplant experiment. Bone marrow derived from *Smo*^{Null} or *Smo*^{WT} mice was transplanted at various ratios (see Methods) together with a competitor wildtype bone marrow from B6.SJL F1 into lethally irradiated B6.SJL recipients (n=15 per group). Mice were treated with i.p. pIpC (25 μg/gram body weight) 3 weeks after transplantation. Peripheral blood DNA was analyzed 4 weeks after pIpC injection to confirm *Smo* excision. L = molecular weight markers, Lanes 1-3 = *Smo*^{Null} samples, Lanes 4-6 = *Smo*^{WT} samples.

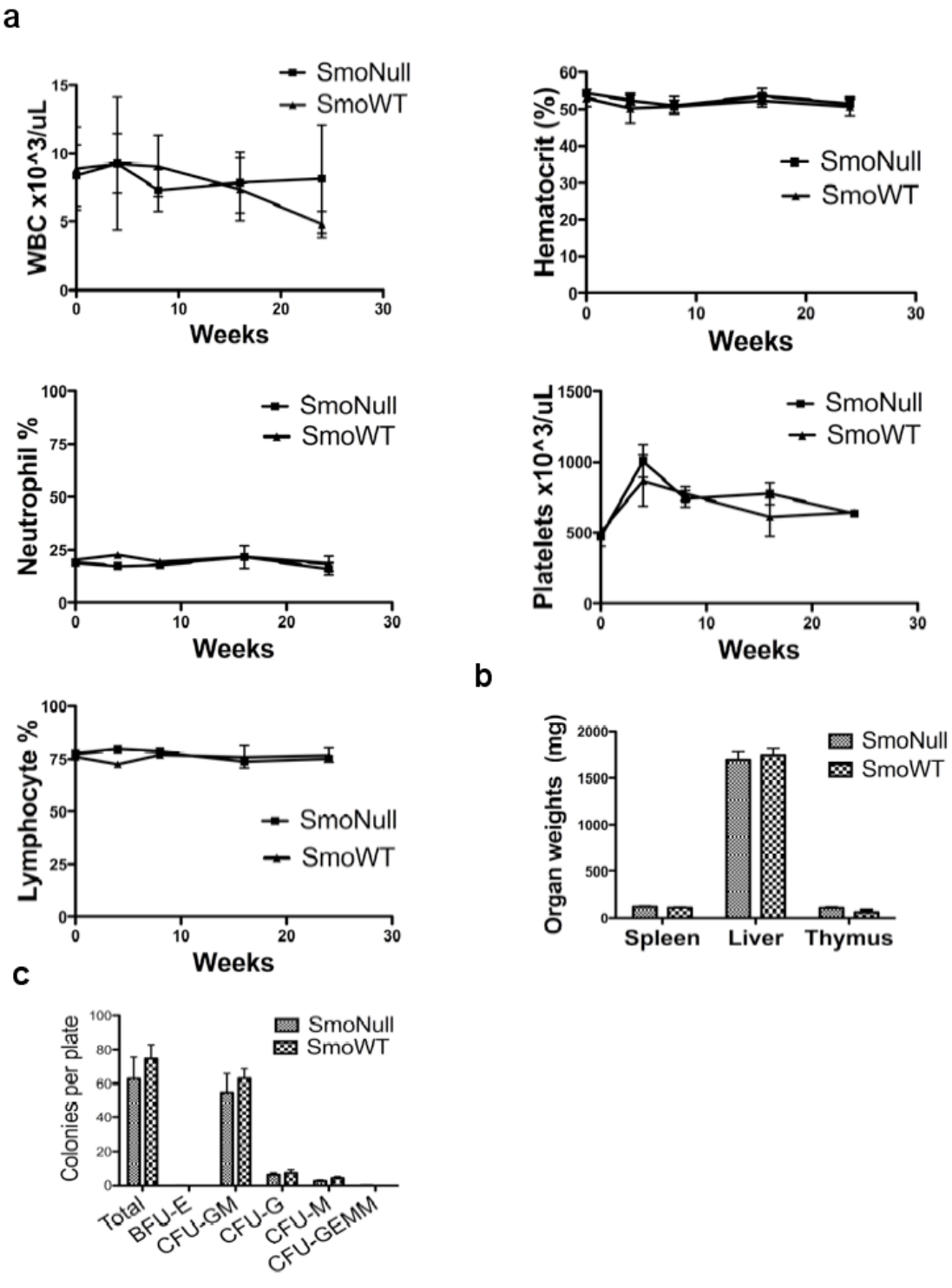
a
SmoNull
SmoWT
WBC x10^3/uL
Weeks
Hematocrit (%)
Neutrophil %
Platelets x10^3/uL
Lymphocyte %
b
Organ weights (mg)
Spleen
Liver
Thymus
c
Colonies per plate
Total
BFU-E
CFU-GM
CFU-G
CFU-M
CFU-GEMM

Figure S2. Loss of *Smo* does not affect peripheral blood counts, organ weights, or CFU potential.

(a) Smo^{Null} or Smo^{WT} mice (n=3-6 per group) were treated with 25 μg/gram pIpC by i.p. injection at 10 weeks of age. Peripheral blood counts were obtained at 4, 8, 18 and 24 weeks for analysis. No difference in WBC (top left), % neutrophil count (middle left), % lymphocyte count (bottom left), hematocrit (top right), or platelet count (lower right) was observed (p=0.1292-0.735, unpaired *t* test). Error bars represent s.d. (b) Same experiment as (a) demonstrating no effect on spleen, liver, and thymus weight at the end point analysis 6 months after pIpC injection (p=0.4480, 0.1823, and 0.7187, unpaired *t* test). Error bars represent s.e.m. (c) No difference in the CFU potential of Smo^{Null} (n=10) vs Smo^{WT} (n=4) mice 18 months after pIpC treatment (p=0.8393, unpaired *t* test). Error bars represent s.e.m.

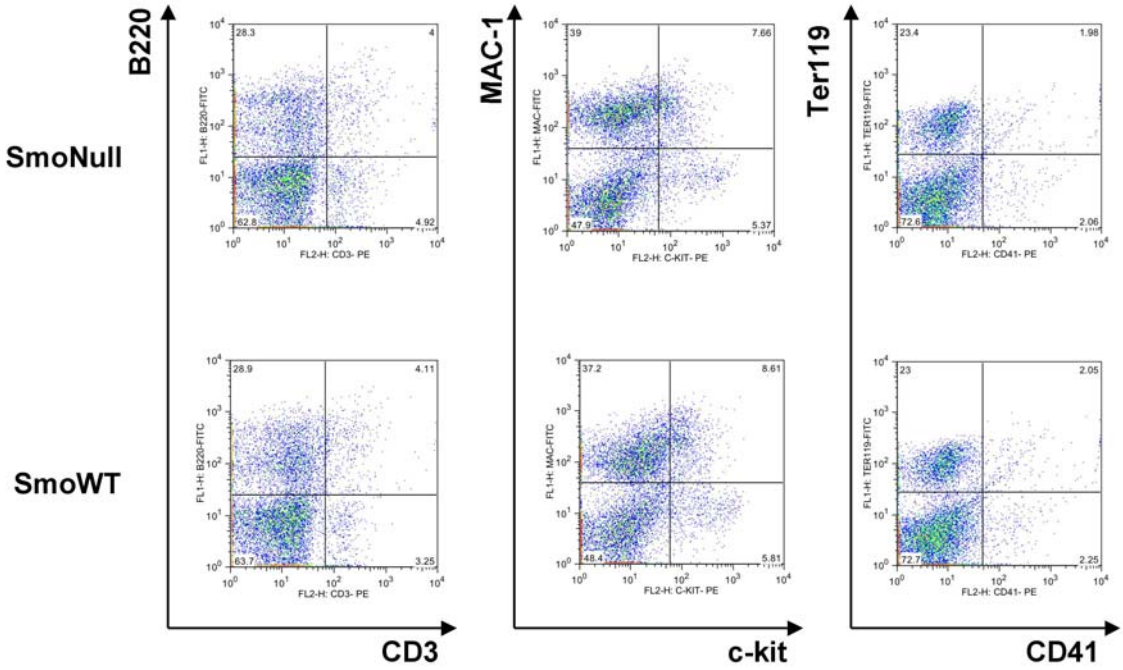

Figure S3. Loss of *Smo* shows no effect on terminally differentiated cells.

Same experiment as **Figure S1b**. Representative flow analysis panels are shown for Smo^{Null} (top row) and Smo^{WT} (bottom row) mice. Loss of Smo did not lead to any differences in lymphocytes as shown by CD3 positive T cells and B220 positive B cells (left); myeloid cells as shown by expression of Mac-1 and c-kit (middle); or Ter119 positive erythroid cells and CD41 positive megakaryocytes.