Qian et al., http://www.jem.org/cgi/content/full/jem.20080578/DC1

***Apc* mutant mice.** *Mx1-Cre$^+$Apc$^{fl/+}$* mice, generated by crossing *Apc$^{fl/fl}$* mice (1) with *Mx1-Cre* transgenic mice (2), were crossed with *Apc$^{fl/fl}$* mice to generate *Mx1-Cre$^+$Apc$^{fl/fl}$*, *Mx1-Cre$^−$Apc$^{fl/fl}$*, *Mx1-Cre$^+$Apc$^{fl/+}$*, and *Mx1-Cre$^−$Apc$^{fl/+}$* mice. Genotyping was performed by PCR analysis of tail DNA using primers P3 (5′-GTTCTGTATCATGGAAAGATAGGTGGTC-3′), P4 (5′-CACTCAAAACGCTTTTGAGGGTTGATTC-3′), and P5 (5′-GAGTACG-GGGTCTCTGTCTCAGTGAA-3′; reference 1). To induce deletion of exon 14 of *Apc*, mice were injected i.p. with 6–10 µg/gram body weight of pI-pC two or three times every 2 d. The efficiency of deletion in hematopoietic cells was verified by PCR using primers P3 and P4. The expression of *Apc* in HSCs after induction of *Apc* deletion was analyzed by qRT-PCR using primers A (5′-ACAAGACGGCAGCTGGAGTATGAA-3′) and B (5′-TGGATCCTG-GCTATTCTTCGCTGT-3′). Mice at 2 mo of age or older were used for experiments. The chimeric mice were analyzed 2 mo after transplantation.

Histology and peripheral blood analyses. Sternum obtained from mice were fixed and decalcified in Cal-Rite (Richard-Allan Scientific), embedded, sectioned, and stained with hematoxylin and eosin. Blood smears were stained in May-Gruenwald-Giemsa. Peripheral blood was collected by tail bleeding. The WBC, red blood cell, and platelet counts, hemoglobin level, and WBC differentials were determined with a Hemavet counter (CDC Technologies, Inc.).

CAFC assay. The CAFC assay was performed as previously described (3). In brief, FBMD-1 stromal cells were cultured in 96-well plates for 10–14 d to form confluent monolayers. Total BM cells were isolated from *Mx1-Cre$^+$Apc$^{fl/fl}$* or *Mx1-Cre$^−$Apc$^{fl/fl}$* mice 4 d after two pI-pC injections and plated at dilutions of 81,000, 27,000, 9,000, 3,000, 1,000, and 333 cells per well with 20 replicate wells onto the monolayer of FBMD-1 stromal cells. Cobblestone areas, defined as a colony of at least five cells underneath the stroma, were scored at day 35. The frequency of CAFC was calculated using the maximum likelihood method.

Homing Assays. This experiment was performed as previously described (4). At 4 d after induction, the *Apc* mutant and control BM cells were isolated and labeled with CFSE (Invitrogen) as per the manufacturer's instructions. The labeled *Apc* control or *Apc* mutant BM cells were transplanted into lethally irradiated recipient mice (2×10^6 or 6×10^6 per mouse, respectively) by retroorbital injection. The proportion of CFSE-labeled cells in BM of recipient mice was examined by flow cytometry, 6 h after injection. PCR analysis of genomic DNA from the BM cells was performed to confirm deletion of the floxed *Apc* alleles.

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Table S1. Primers used in qRT–PCR analysis

Primer name	Sequence
Birc5-f	TCTGGCAGCTGTACCTCAAGAACT
Birc5-r	CCCAGCCTTCCAATTCCTTAAAGC
Cdkn1a-f	ACCAGCCTGACAGATTTCTA
Cdkn1a-r	TGACCCACAGCAGAAGAG
Myc-f	CTGTTTGAAGGCTGGATTT
Myc-r	TCGAGGTCATAGTTCCTGTT
Cdkn1b-f	GTGGACCAAATGCCTGACTCGT
Cdkn1b-r	GGGCTTCTTGGGCGTCTGCT
Bcl2-f	TTGTGGCCTTCTTTGAGTTCGGTG
Bcl2-r	CTTCAGAGACAGCCAGGAGAAATC
Tp53-f	AAAGGATGCCCATGCTACAGAGGA
Tp53-r	ATGGGAGCTAGCAGTTTGGGCTTT
Gata1-f	GCACTAACTGTCAAACGACCAC
Gata1-r	CGTCTGGATTCCATCTTTCC
Myb-f	GAATCATTTACCAGGCACAC
Myb-r	CCAGTGGTTCTTGATAGCAT
Gata2-f	CAACCCTTACTACGCCAAC
Gata2-r	CTGTGCAACAAGTGTGGTC
Bmi1-f	ACCTGGAGAAGAAATGGCCCACTA
Bmi1-r	AGTCACTTTCCAGCTCTCCAGCAT
Mcl1-f	GGTGCCTTTGTGGCCAAACACTTA
Mcl1-r	ACGTGGAAGAACTCCACAAACCCA
Hoxb4-f	GCACGGTAAACCCCAATTA
Hoxb4-r	GGCAACTTGTGGTCTTTTTT
Ccnd1-f	TGCTGCAAATGGAACTGCTTCTGG
Ccnd1-r	TACCATGGAGGGTGGGTTGGAAAT
Bcl2l1-f	AAGCGTAGACAAGGAGATGCAGGT
Bcl2l1-r	CTGCTGCATTGTTCCCGTAGAGAT
Tnfrsf1a-f	TCAGGCAGTGTCTCAGTTGCAAGA
Tnfrsf1a-r	ACTGGTTCTCCTTACAGCCACACA

Table S2. Mean threshold cycle (C$_t$) value for each gene in qRT–PCR analysis

Gene	Mean Ct value	
	Mx1-Cre⁻Apc^{fl/fl}	*Mx1-Cre⁺Apc^{fl/fl}*
Hprt	35.13911206	37.51221983
Ccnd1	36.01570817	37.50281217
Cdkn1b	37.84073444	42.02595556
Cdkn1a	31.01984411	34.02924311
Myc	27.726358	30.87373056
Myb	26.31670022	29.42454544
Bmi1	30.55012544	33.39295278
Gata2	28.78074422	31.79048467
Gata1	33.16550728	35.28810883
Hoxb4	34.23637756	36.65977617
Tnfrsf1a	29.11553489	32.40085978
Mcl1	28.58722011	31.69094411
Tp53	32.02020944	33.55653322
Birc5	34.871307	36.40605422
Bcl2l1	34.28690556	36.86379217
Bcl2	33.47593678	36.42065089