ement and overtranscription remains to be proven. Fourth, and finally, earlier work on amplified esterase genes in mosquitoes suggested that a single global spread of one specific amplicon accounted for insecticide resistance in global populations of *Culex pipiens* mosquitoes (*30*). Further analysis of mosquito populations, however, showed that numerous different mutational events and their resulting amplicons make up the extant global population of resistance alleles in mosquitoes (*24*, *31*). Our description of an identical resistant allele in 20 DDT-resistant strains of *D. melanogaster* of diverse geographic origin represents the global spread of a single insecticide-resistance allele.

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Supporting Online Material
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Table S1

20 May 2002; accepted 19 July 2002

Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells

Amy J. Wagers,* Richard I. Sherwood, Julie L. Christensen, Irving L. Weissman

To rigorously test the in vivo cell fate specificity of bone marrow (BM) hematopoietic stem cells (HSCs), we generated chimeric animals by transplantation of a single green fluorescent protein (GFP)–marked HSC into lethally irradiated nontransgenic recipients. Single HSCs robustly reconstituted peripheral blood leukocytes in these animals, but did not contribute appreciably to nonhematopoietic tissues, including brain, kidney, gut, liver, and muscle. Similarly, in GFP$^+$:GFP$^-$ parabiotic mice, we found substantial chimerism of hematopoietic but not nonhematopoietic cells. These data indicate that "transdifferentiation" of circulating HSCs and/or their progeny is an extremely rare event, if it occurs at all.

As many recent reports have suggested that BM HSCs may harbor unexpected developmental plasticity (*1–14*), we set out to test rigorously the cell fate potential of prospectively isolated, long-term reconstituting HSCs (*15–17*) using chimeric animals generated by transplantation of a single GFP$^+$ c-kit$^+$Thy1.1^{lo}Lin$^-$Sca-1$^+$ (KTLS) BM HSC (fig. S1) (*18*). GFP$^+$ HSCs were isolated by fluorescence-activated cell sorting (FACS) from BM of transgenic animals that constitutively express GFP, driven by the β-actin promoter, in all tissues (*19*). About 18% of recipients of single GFP$^+$ KTLS HSCs showed significant levels of long-term, multilineage (both lymphoid and myeloid) hematopoietic engraftment in the peripheral blood (Table 1). Although the hematopoietic contribution from single GFP$^+$ HSCs varied, in some recipients donor-derived contributions reached levels as high as ~70% (Table 1).

To further evaluate the cell fate potential of transplanted HSCs, we analyzed tissues from engrafted recipients 4 to 9 months after transplant for the presence of GFP$^+$ cells by standard and confocal fluorescence microscopy (*18*). Tissues from recipient animals exhibiting multilineage reconstitution of GFP$^+$ blood leukocytes were stained with tissue-specific antibodies and/or with the pan-hematopoietic marker, CD45 (*20–22*). These sections were then analyzed to identify potentially transdifferentiated GFP$^+$ cells, which satisfied one or more of the following criteria: (i) the GFP$^+$ cell stains with tissue-specific markers; (ii) the GFP$^+$ cell does not stain with a monoclonal antibody to CD45; and (iii) the cell exhibits distinctive morphology, indicative

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Table 1. Frequency and degree of reconstitution in single HSC–transplanted mice. The peripheral blood (PB) of single HSC–reconstituted mice was analyzed by flow cytometry 5 and 14 weeks after transplant for the presence of GFP$^+$ donor-derived leukocytes. PB cells were stained for markers of the lymphoid (L) lineage (CD3$^+$,B220$^+$) versus myeloid (M) lineage (Mac-1$^+$, Gr-1$^+$) or separately for B cells (B, B220$^+$), T cells (T, CD3$^+$), and myeloid cells (M, Mac-1$^+$, Gr-1$^+$).

Frequency of reconstitution (reconstituted mice/total)	
5 weeks	14 weeks
7/22 (32%) L + M	4/22 (18%) BTM
5/22 (23%) L only	1/22 (5%) BT
	2/22 (9%) B only
Average reconstitution (% GFP$^+$ PB leukocytes)	
17.6% (range: 0.12–77.6%)	20.2% (range: 0.03–71.6%)

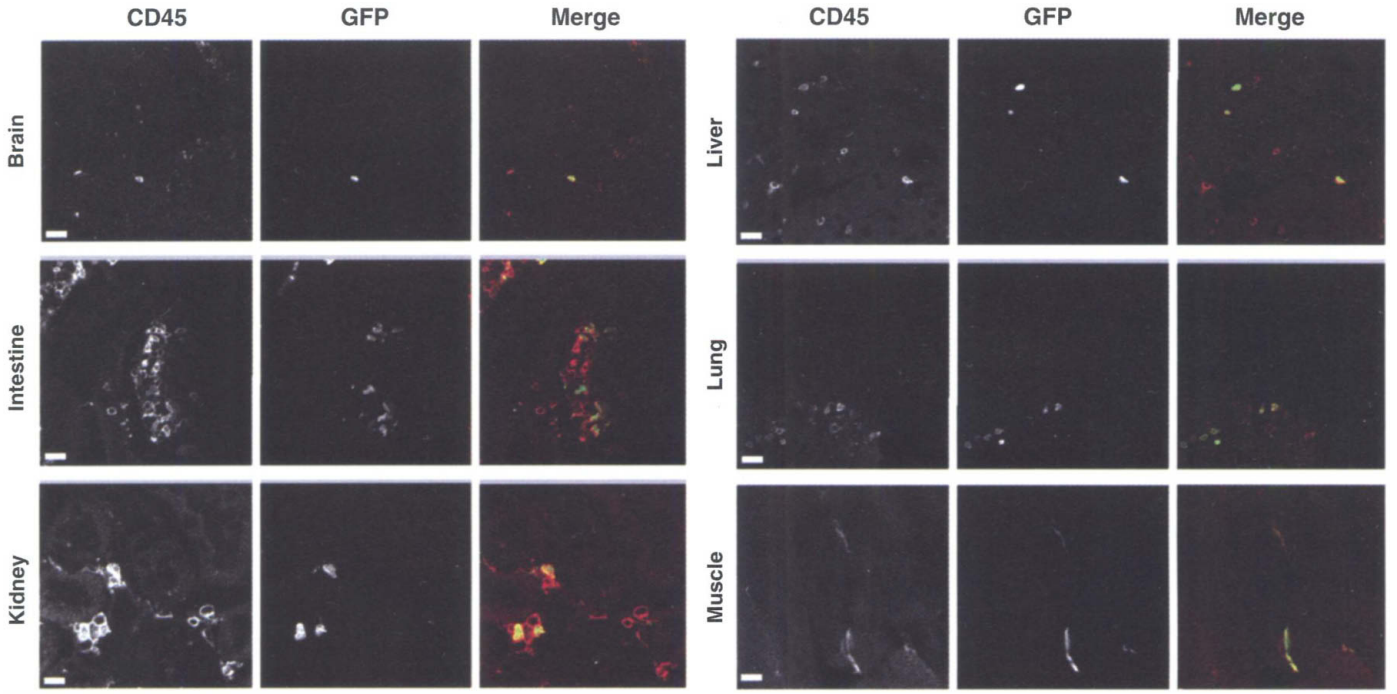

Fig. 1. GFP$^+$ cells in the tissues of nontransgenic recipients of single GFP$^+$ stem cells. All recipients analyzed showed robust, multilineage engraftment of the hematopoietic compartment (not shown). Representative confocal micrographs from brain (bar, 20 μm), intestine (bar, 20 μm), kidney (bar, 8 μm), liver (bar, 20 μm), lung (bar, 20 μm), and skeletal muscle (bar, 20 μm) are shown. The left column shows anti-CD45 staining, the middle column shows GFP expression, and the right column shows the merged image (GFP expression is shown in green, and anti-CD45 is shown in red).

Table 2. Analysis of HSC-derived cells in single HSC–transplanted mice. Frozen sections of the indicated tissues from single GFP$^+$ HSC–transplanted mice were analyzed for the presence of "transdifferentiated" cell types (*18*). The criteria for the identification of GFP$^+$ nonhematopoietic cells were as given in the text.

Tissue	No. of sections examined	~No. of cells examined	No. of GFP$^+$ nonhematopoietic cells
Brain	60	13,200,000	1
Liver	18	470,000	7
Kidney	24	990,000	0
Gut	24	360,000	0
Skeletal muscle	23	2,355	0
Cardiac muscle	14	4,346	0
Lung	12	23,000	0

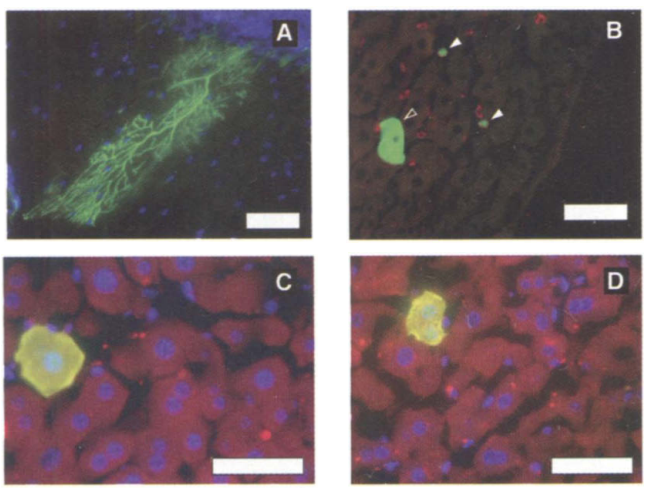

Fig. 2. HSC-derived nonhematopoietic cells in single HSC–transplanted mice. (**A**) GFP$^+$ Purkinje cell in the brain of a nontransgenic recipient of a single GFP$^+$ HSC. Nuclear labeling with Hoechst dye is shown in blue and GFP expression is shown in green (bar, 50 μm). Of three independently transplanted mice, we observed only one GFP$^+$ neuronal cell. (B to D) GFP$^+$ hepatocytes in the liver of nontransgenic recipients of a single GFP$^+$ HSC. (**B**) GFP$^+$ CD45$^-$ hepatocyte (open arrowhead) and two GFP$^+$CD45$^+$ hematopoietic cells (closed arrowheads) are noted. GFP expression is shown in green, and anti-CD45 staining is shown in red (bar, 50 μm). (**C** and **D**) Nuclear labeling with Hoechst dye is shown in blue, GFP expression is in green, and anti-albumin reactivity is in red. Yellow color indicates colocalization of GFP and albumin (bar, 50 μm). In two independently transplanted mice, we observed seven GFP$^+$ hepatocytes.

of a differentiated, nonhematopoietic cell fate. The results of these analyses are summarized in Table 2, and representative micrographs are shown in Fig. 1. Although single HSCs contributed substantially to the generation of mature hematopoietic cells, which were readily detected in all tissues (Fig. 1 and Table 1), most tissues showed no evidence of GFP$^+$ nonhematopoietic cells. In particular, we found no GFP$^+$ cells that fulfilled the above-defined criteria for transdifferentiation in lung, kidney, gut, or muscle (Table 2), and the vast majority of GFP$^+$ cells in all tissues expressed the pan-hematopoietic marker CD45 (Fig. 1). Furthermore, GFP$^+$ cells in muscle did not stain for the muscle-specific markers α-actinin or dystrophin, GFP$^+$ cells in lung and intestine did not stain for the epithelium-specific marker pan-cytokeratin, GFP$^+$ cells in kidney did not stain with the lectin wheat-germ agglutinin (WGA), and GFP$^+$ cells in the brain did not express the neuronal marker MAP-2 (*23*). Among 60 sagittal sections of brain analyzed, we identified only one GFP$^+$ nonhematopoietic cell, a Purkinje cell (Fig. 2A). Single transplanted HSCs also contributed at a very low level to the production of liver hepatocytes. These cells were identified at a frequency of ~1 in 70,000 liver cells, did not express CD45, and did express the hepatocyte marker albumin (Table 2 and Fig. 2). GFP$^+$ hepatocytes always appeared as individual, isolated cells, unlike the distinct nodules previously observed by Lagasse and colleagues following transplantation of 50 to 1000 KTLS HSCs into fumarylacetoacetate hydrolase (FAH)–deficient mice (*9*).

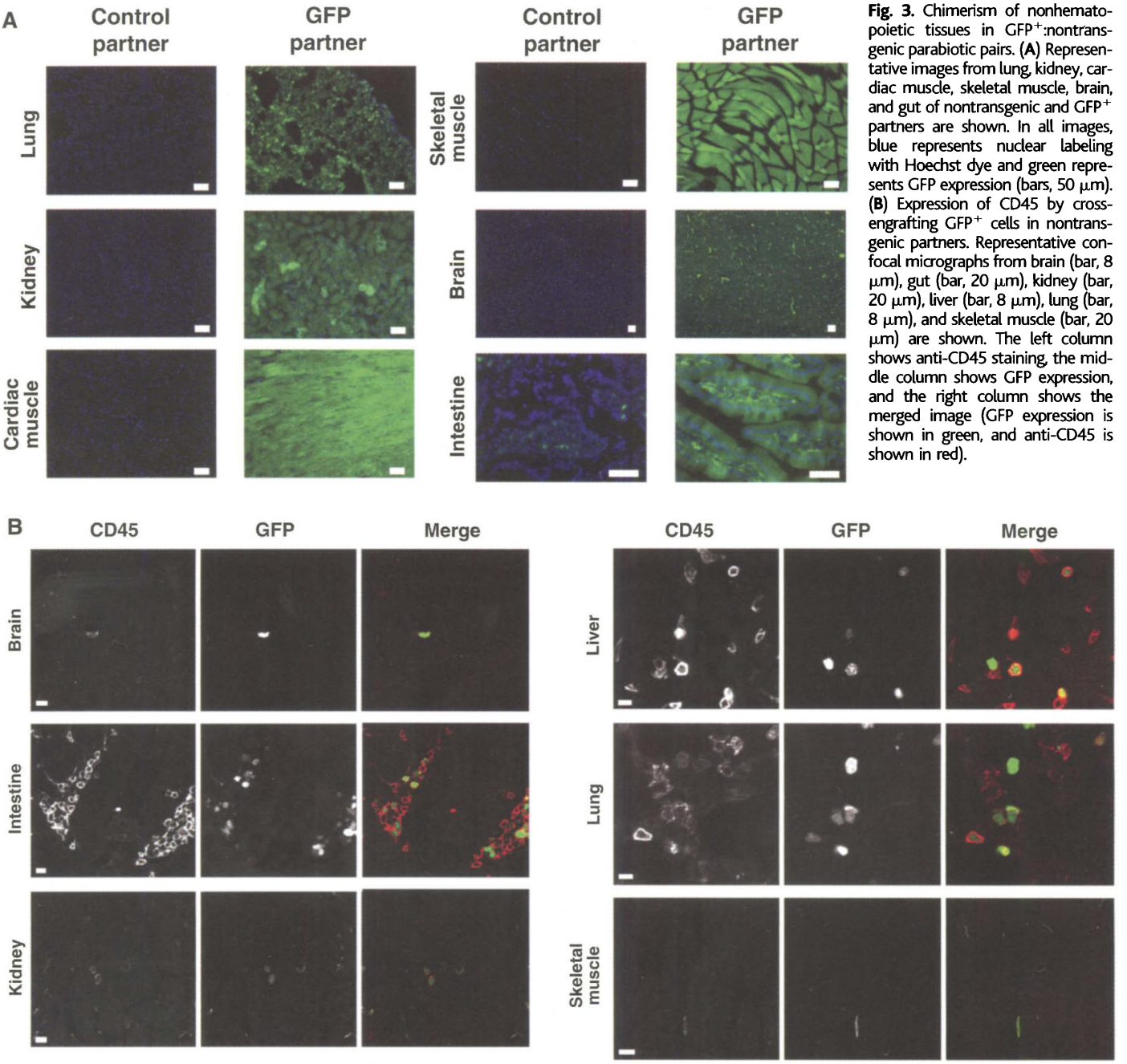

Fig. 3. Chimerism of nonhematopoietic tissues in GFP$^+$:nontransgenic parabiotic pairs. (**A**) Representative images from lung, kidney, cardiac muscle, skeletal muscle, brain, and gut of nontransgenic and GFP$^+$ partners are shown. In all images, blue represents nuclear labeling with Hoechst dye and green represents GFP expression (bars, 50 μm). (**B**) Expression of CD45 by cross-engrafting GFP$^+$ cells in nontransgenic partners. Representative confocal micrographs from brain (bar, 8 μm), gut (bar, 20 μm), kidney (bar, 20 μm), liver (bar, 8 μm), lung (bar, 8 μm), and skeletal muscle (bar, 20 μm) are shown. The left column shows anti-CD45 staining, the middle column shows GFP expression, and the right column shows the merged image (GFP expression is shown in green, and anti-CD45 is shown in red).

Thus, these data indicate that transdifferentiation of HSCs into nonhematopoietic cell fates is not a common outcome of the HSC developmental program, strongly suggesting that if there are cells with extensive capacity for nonhematopoietic cell differentiation resident in the BM, they are likely not HSCs. Such activity may arise from the presence in the marrow of distinct, tissue-specific stem cells; or from a marrow-resident pluripotent or multipotent stem cell (*24*, *25*); or from a rare fusion event between a differentiated nonhematopoietic cell and a hematopoietic cell that transits through the tissue (*26*, *27*). In this regard, it is of interest that liver hepatocytes, in which HSC-derived contributions were detected in single HSC–transplanted mice, may normally exhibit hyperdiploid nuclear DNA content (*28*).

Like their more mature progeny, normal murine HSCs constitutively circulate in the bloodstream, and circulating HSCs are fully capable of reseeding hematopoiesis at distant BM sites (*29*, *30*). However, whether HSCs or other circulating lineage-specific or multipotent progenitor cells make similar contributions to the generation of nonhematopoietic tissues in normal animals has remained unclear. To investigate this possibility, we examined tissue chimerism of parabiotic animals, which are surgically joined such that they rapidly develop a common, anastomosed circulatory system (*18*, *31*), allowing the evaluation in this model system of potential regenerative contributions by any circulating stem/progenitor cell, including HSCs, which may use the blood as a conduit to access various tissues. The parabiotic system also provides a mechanism for HSC engraftment that does not require lethal irradiation (or the tissue damage associated with it) to ablate the host's hematopoietic system. Using parabiotic pairs joined for 6 to 7 months and containing one GFP transgenic and one nontransgenic littermate, we examined the cross-engraftment of partner cells in both hematopoietic and nonhematopoietic tissues (*18*). Hematopoietic chimerism in parabiotic blood and spleen is detectable within 2 to 3 days of joining and reaches equilibrium by days 8 to 10 (*30*). BM equil-

Table 3. Chimerism in blood and bone marrow of long-term parabionts. Nontransgenic (nonTg) and GFP$^+$ mice were surgically joined for 6 (Pair 3) or 7 (Pairs 1 and 2) months. Hematopoietic cell chimerism in the blood and bone marrow (BM) was determined by flow cytometric analysis and is given as the % GFP$^+$ cells in the nonTg partner and %GFP$^-$ cells in the GFP$^+$ partner. Chimerism of BM HSC was determined after staining of BM cells for c-kit, Thy1.1, lineage, and Sca-1 (KTLS).

	nonTg partner			GFP$^+$ partner			
	Pair 1	Pair 2	Pair 3	Pair 1	Pair 2	Pair 3	Average chimerism ± SD
Blood	22.1	71.2	83.4	78.4	28.2	10.4	49.0 ± 32.2
BM	4.69	73	19.2	81.3	14.4	7.82	33.4 ± 34.4
BM HSCs	24.9	69.4	10.5	27.2	11.4	2	24.2 ± 24.1

Table 4. Analysis of partner-derived GFP$^+$ cross-engrafting cells in parabiotic mice. Frozen sections of the indicated tissues from nontransgenic partners of parabiotic pairs were analyzed for the presence of "transdifferentiated" cell types (*18*). The criteria for the identification of partner-derived GFP$^+$ nonhematopoietic cells were as given in the text.

Tissue	No. of sections examined	~No. of cells examined	No. of partner-derived nonhematopoietic cells
Brain	30	3,189,000	0
Liver	9	174,100	0
Kidney	8	101,000	0
Gut	8	399,000	0
Skeletal muscle	32	3,037	0
Cardiac muscle	10	2,014	0
Lung	6	226,000	0

ibration occurs more slowly, but cross-engraftment of functional HSCs is detectable by 2 to 3 weeks after joining (fig. S2) and can be quite substantial in parabionts joined for several months (Table 3) (*18*). However, despite high levels of hematopoietic cross-engraftment, analysis of multiple tissues in long-term parabionts revealed no evidence for engraftment of nonhematopoietic tissues by circulating HSCs or other tissue-specific cells (Fig. 3 and Table 4). Thus, steady-state tissue regeneration appears to derive predominantly from tissue-resident progenitor cells, rather than from circulating cells. The lack of cross-engrafting nonhematopoietic cells in parabionts further supports our findings in single cell–transplanted mice, because parabiotic animals are exposed to a constant, albeit low-level, source of HSCs, which should be capable of contributing to nonhematopoietic cell fates if in fact this potential is a true and robust property of HSCs.

Thus, taken together, the data generated by these two complementary experimental approaches provide strong evidence against the hypothesis that BM KTLS HSCs, the only HSCs in BM as assayed by transplantation (*32*), possess a robust, intrinsic capacity for the production of nonhematopoietic cells. However, these data cannot rule out the potential of HSCs to be recruited into atypical functions in the face of severe injury and/or selective pressure. The observation that as few as 50 highly purified HSCs can be induced to generate large colonies of functional hepatocytes when introduced into FAH$^{-/-}$ mice (*9*)—although only a minor contribution to hepatopoiesis is detected in transplanted animals that do not suffer FAH deficiency (Table 4)—suggests that strong selective pressure may facilitate HSC-derived hepatopoiesis. Such selection could promote the generation of FAH$^+$ cells, whether they arise by transdifferentiation or by cell fusion with endogenous hepatocyte progenitors,by rescuing host cells with donor-derived enzymes.

To address the possibility that tissue injury may recruit HSCs or their progeny into nonhematopoietic cell fates, we evaluated HSC-derived contributions to epithelial cell regeneration following irradiation-induced intestinal injury. Single HSC–transplanted animals were subjected to localized x-irradiation (abdominal cavity only) (*18*), which causes recoverable destruction of radiosensitive intestinal epithelial stem cells and shrinkage of intestinal villi (*33–35*). The ability of HSCs or their progeny to contribute to the ensuing regeneration of intestinal epithelium was then evaluated, using the criteria described earlier. CD45$^+$ GFP$^+$ cells were readily detected in the injured intestine (fig. S3); however, we observed no GFP$^+$ intestinal epithelial cells among ~640,000 cells from 36 different sections examined, suggesting that, at least for this tissue type, acute injury does not induce HSC plasticity. Thus, although the capacity of other selective models to elicit HSC plasticity in other organ systems must still be determined, our results clearly demonstrate that the production of nonhematopoietic cell types is not a typical function of normal HSCs.

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