

## Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells

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**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<sup>+</sup>:GFP<sup>-</sup> parabiotic mice, we found significant 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 HSC may harbor unexpected developmental plasticity (1–14), we set out to test rigorously the cell fate potential of prospectively isolated, long-term reconstituting HSC (15–17) using chimeric animals generated by transplantation of a single GFP<sup>+</sup> c-kit<sup>+</sup>Thy1.1<sup>+</sup>Lin<sup>-</sup>Sca-1<sup>+</sup> (KTLS) BM HSC (Figure S1) (18). GFP<sup>+</sup> HSC were isolated by FACS sorting from BM of transgenic animals that constitutively express GFP, driven by the β-actin promoter, in all tissues (19). ~18% of recipients of single GFP<sup>+</sup> KTLS HSC showed significant levels of long-term, multi-lineage (both lymphoid and myeloid) hematopoietic engraftment in the peripheral blood (Table 1). Although the hematopoietic contribution from single GFP<sup>+</sup> HSC varied, in some recipients donor-derived contributions reached levels as high as ~70% (Table 1).

To further evaluate the cell fate potential of transplanted HSC, we analyzed tissues from engrafted recipients 4–9 months after transplant for the presence of GFP<sup>+</sup> cells by standard and confocal fluorescence microscopy (18). Tissues from recipient animals exhibiting multi-lineage reconstitution of GFP<sup>+</sup> 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<sup>+</sup> cells, which satisfied one or more of the following criteria: (i) the GFP<sup>+</sup> cell stains with tissue-specific markers; (ii) the GFP<sup>+</sup> cell does not stain with anti-CD45 mAb; (iii) the cell exhibits distinctive morphology, indicative of a differentiated, non-hematopoietic cell fate. The results of these analyses are summarized in Table 2, and representative micrographs are shown in Figure 1. While single HSC made significant contributions to the generation of mature hematopoietic cells, which were readily detected in all tissues (Figure 1, Table 1), the majority of tissues showed no evidence of GFP<sup>+</sup> non-hematopoietic cells. In particular, we found no GFP<sup>+</sup> cells which fulfilled the above-defined criteria for transdifferentiation in lung, kidney, gut, or muscle

(Table 2), and the vast majority of GFP<sup>+</sup> cells in all tissues expressed the pan-hematopoietic marker, CD45 (Figure 1). Furthermore, GFP<sup>+</sup> cells in muscle did not stain for the muscle-specific markers α-actinin or dystrophin, GFP<sup>+</sup> cells in lung and intestine did not stain for the epithelium-specific marker pan-cytokeratin, GFP<sup>+</sup> cells in kidney did not stain with the lectin wheat germ agglutinin (WGA), and GFP<sup>+</sup> cells in the brain did not express the neuronal marker MAP-2 (data not shown). Among 60 sagittal sections of brain analyzed, we identified only one GFP<sup>+</sup> non-hematopoietic cell, a Purkinje cell (Figure 2A). Single transplanted HSC 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, Figure 2). GFP<sup>+</sup> hepatocytes always appeared as individual, isolated cells, unlike the distinct nodules previously observed by Lagasse and colleagues following transplantation of 50–1000 KTLS HSC into fumarylacetoacetate hydrolase (FAH)-deficient mice (9).

Thus, these data indicate that transdifferentiation of HSC into non-hematopoietic cell fates is not a common outcome of the HSC developmental program, strongly suggesting that if there are cells with extensive capacity for non-hematopoietic cell differentiation resident in the bone marrow, they are likely not HSC. 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 (23, 24); or, from a rare fusion event between a differentiated non-hematopoietic cell and a mature hematopoietic cell that transits through the tissue (25, 26). In this regard, it is interesting to note that liver hepatocytes, in which HSC-derived contributions were detected in single HSC transplanted mice, may normally exhibit hyperdiploid nuclear DNA content (27).

Like their more mature progeny, normal murine HSC constitutively circulate in the bloodstream, and circulating HSC are fully capable of re-seeding hematopoiesis at distant BM sites (28, 29). However, whether HSC or other circulating lineage-specific or multipotent progenitor cells make similar contributions to the generation of non-hematopoietic 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, 30), allowing the evaluation in this model system of potential regenerative contributions by any circulating stem/progenitor cell, including HSC, 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 6–7 months and containing one GFP transgenic and one non-transgenic littermate, we examined the cross-engraftment of partner cells in both hematopoietic and non-hematopoietic tissues (18). Hematopoietic chimerism in parabiotic blood and spleen is detectable within 2–3 days of joining, and reaches equilibrium by days 8–10 (29). BM equilibration occurs more slowly, but cross-engraftment of functional HSC is detectable by 2–3 weeks after joining (Figure 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 of engraftment of non-hematopoietic tissues by circulating HSC or other tissue-specific cells (Figure 3, Table 4). Thus, steady-state tissue regeneration appears to derive predominantly from tissue resident progenitor cells, rather than circulating cells. The lack of cross-engrafting non-hematopoietic cells in parabionts further supports our findings in single cell transplanted mice, as parabiotic animals are exposed to a constant, albeit low-level, source of HSC, which should be capable of contributing to non-hematopoietic cell fates, if in fact this potential is a true and robust property of HSC.

Thus, taken together, the data generated by these two complementary experimental approaches strongly argue against the hypothesis that BM KTLS HSC, the only HSC in BM as assayed by transplantation (31), possess a robust, intrinsic capacity for the production of non-hematopoietic cells. However, these data cannot rule out the potential of HSC 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 HSC can be induced to generate large colonies of functional hepatocytes when introduced into FAH<sup>−/−</sup> mice (9), although only minor contribution to hepatogenesis is detected in transplanted animals which do not suffer FAH deficiency (Table 4), suggests that strong selective pressure may facilitate HSC-derived hepatogenesis. Such selection could promote the generation of FAH<sup>+</sup> cells, whether they arise by transdifferentiation or by cell fusion with endogenous hepatocyte progenitors, by rescuing host cells with donor-derived enzymes.

To begin to address the possibility that tissue injury may recruit HSC or their progeny into non-hematopoietic 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 (32–34). The ability of HSC or their progeny to contribute to the ensuing regeneration of intestinal epithelium was then evaluated, using the criteria described earlier. CD45<sup>+</sup> GFP<sup>+</sup> cells were easily detected in the injured intestine (Figure S3); however, we observed no GFP<sup>+</sup> 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 non-hematopoietic cell types is not a typical function of normal HSC.

## References and Notes

- E. Gussoni *et al.*, *Nature* **401**, 390 (1999).
- G. Ferrari *et al.*, *Science* **279**, 1528 (1998).
- M. A. Eglitis, E. Mezey, *Proc Natl Acad Sci U S A* **94**, 4080 (1997).
- T. R. Brazelton, F. M. Rossi, G. I. Keshet, H. M. Blau, *Science* **290**, 1775 (2000).
- E. Mezey, K. J. Chandross, G. Harta, R. A. Maki, S. R. McKercher, *Science* **290**, 1779 (2000).
- D. Orlic *et al.*, *Ann N Y Acad Sci* **938**, 221 (2001).
- D. Orlic *et al.*, *Proc Natl Acad Sci U S A* **98**, 10344 (2001).
- B. E. Petersen *et al.*, *Science* **284**, 1168 (1999).
- E. Lagasse *et al.*, *Nat Med* **6**, 1229 (2000).
- N. D. Theise *et al.*, *Hepatology* **31**, 235 (2000).
- K. A. Jackson *et al.*, *J Clin Invest* **107**, 1395 (2001).
- D. S. Krause *et al.*, *Cell* **105**, 369 (2001).
- M. Sata *et al.*, *Nat Med* **8**, 403 (2002).
- K. Shimizu *et al.*, *Nat Med* **7**, 738 (2001).
- G. J. Spangrude, S. Heimfeld, I. L. Weissman, *Science* **241**, 58 (1988).
- S. J. Morrison, I. L. Weissman, *Immunity* **1**, 661 (1994).
- S. J. Morrison, A. M. Wandycz, H. D. Hemmati, D. E. Wright, I. L. Weissman, *Development* **124**, 1929 (1997).
- Materials and methods are available as supporting material on *Science* Online.
- D. E. Wright *et al.*, *Blood* **97**, 2278 (2001).
- J. A. Ledbetter, L. A. Herzenberg, *Immunol. Rev.* **47**, 63 (1979).
- W. van Ewijk, P. L. van Soest, G. J. van den Engh, *J. Immunol.* **127**, 2594 (1981).
- P. Johnson, M. A., D. H. W. Ng, Eds., *CD45: A family of leukocyte-specific cell surface glycoproteins* (Blackwell Science, Cambridge, MA, 1997).
- R. E. Schwartz *et al.*, *J Clin Invest* **109**, 1291 (2002).
- Y. Jiang *et al.*, *Nature* **418**, 41 (2002).
- Q. L. Ying, J. Nichols, E. P. Evans, A. G. Smith, *Nature* **416**, 545 (2002).
- N. Terada *et al.*, *Nature* **416**, 542 (2002).
- P. Gerlyng *et al.*, *Cell Prolif* **26**, 557 (1993).
- W. H. Fleming, E. J. Alpern, N. Uchida, K. Ikuta, I. L. Weissman, *Proc Natl Acad Sci U S A* **90**, 3760 (1993).
- D. E. Wright, A. J. Wagers, A. P. Gulati, F. L. Johnson, I. L. Weissman, *Science* **294**, 1933 (2001).
- E. Bunster, R. K. Meyer, *The Anatomical Record* **57**, 339 (1933).
- N. Uchida, I. L. Weissman, *J Exp Med* **175**, 175 (1992).
- H. Quastler, *Radiat Res* **4**, 303 (1956).
- R. F. Hagemann, C. P. Sigdestad, S. Lesher, *Radiat Res* **46**, 533 (1971).
- S. Tsubouchi, T. Matsuzawa, *Radiat Res* **56**, 345 (1973).
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## Supporting Online Material

[www.scienmag.org/cgi/content/full/1074807/DC1](http://www.scienmag.org/cgi/content/full/1074807/DC1)

### Materials and Methods

Figs. S1, S2, S3

### References

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**Fig. 1.** GFP<sup>+</sup> cells in the tissues of non-transgenic recipients of single GFP<sup>+</sup> stem cells. All recipients analyzed showed robust, multilineage engraftment of the hematopoietic compartment (not shown). Representative confocal micrographs from brain (scalebar = 20  $\mu$ m), intestine (scalebar = 20  $\mu$ m), kidney (scalebar = 8  $\mu$ m), liver (scalebar = 20  $\mu$ m), lung (scalebar = 20  $\mu$ m), and skeletal muscle (scalebar = 20  $\mu$ m) 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).

**Fig. 2.** HSC-derived non-hematopoietic cells in single HSC transplanted mice. (A) GFP<sup>+</sup> Purkinje cell in the brain of a non-transgenic recipient of a single GFP<sup>+</sup> HSC. Nuclear labeling with Hoechst dye is shown in blue and GFP expression is shown in green (scalebar = 50  $\mu$ m). Of three independently transplanted mice, we observed only one GFP<sup>+</sup> neuronal cell. (B-D) GFP<sup>+</sup> hepatocytes in the liver of non-transgenic recipients of a single GFP<sup>+</sup> HSC. (B) GFP<sup>+</sup> CD45<sup>-</sup> hepatocyte (open arrowhead) and two GFP<sup>+</sup>CD45<sup>+</sup> hematopoietic cells (closed arrowheads) are noted. GFP expression is shown in green, and anti-CD45 staining is shown in red (scalebar = 50  $\mu$ m). (C, D) Nuclear labeling with Hoechst dye is shown in blue, GFP expression in green, and anti-albumin reactivity in red. Yellow color indicates co-localization of GFP and albumin (scalebar = 50  $\mu$ m). In two independently transplanted mice, we observed 7 GFP<sup>+</sup> hepatocytes.

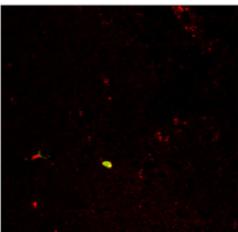
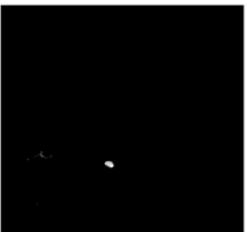
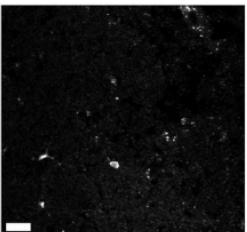
**Fig. 3.** Chimerism of non-hematopoietic tissues in GFP<sup>+</sup>:non-transgenic parabiotic pairs. (A) Representative images from lung, kidney, cardiac muscle, skeletal muscle, brain, and gut of non-transgenic and GFP<sup>+</sup> partners are shown. In all images, blue represents nuclear labeling with Hoechst dye and green represents GFP (scalebars = 50  $\mu$ m). (B) Expression of CD45 by cross-engrafting GFP<sup>+</sup> cells in non-transgenic partners. Representative confocal micrographs from brain (scalebar = 8  $\mu$ m), gut (scalebar = 20  $\mu$ m), kidney (scalebar = 20  $\mu$ m), liver (scalebar = 8  $\mu$ m), lung (scalebar = 8  $\mu$ m), and skeletal muscle (scalebar = 20  $\mu$ m) 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).

**CD45**

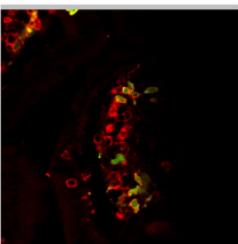
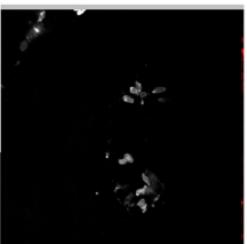
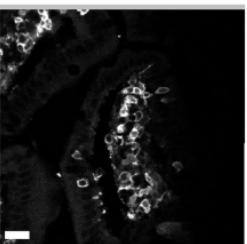
**GFP**

**Merge**

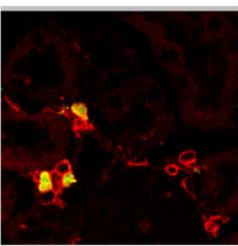
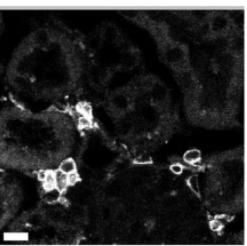
**Brain**



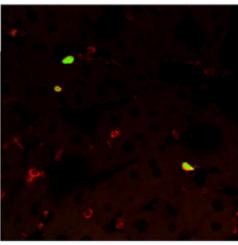
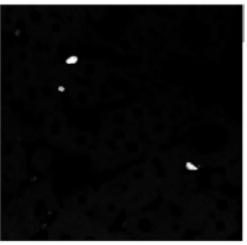
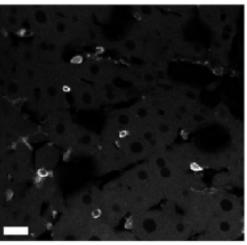
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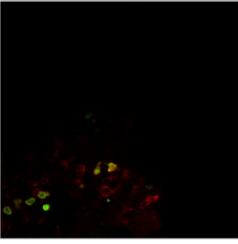
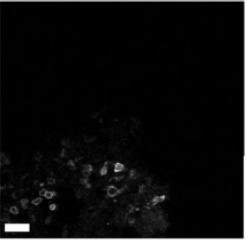
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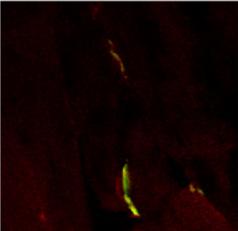
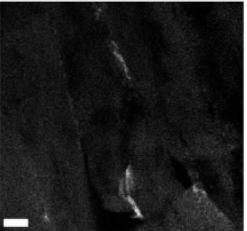
**Liver**

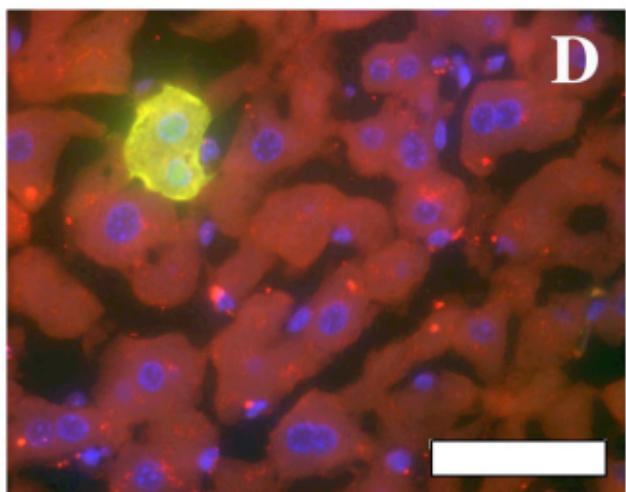
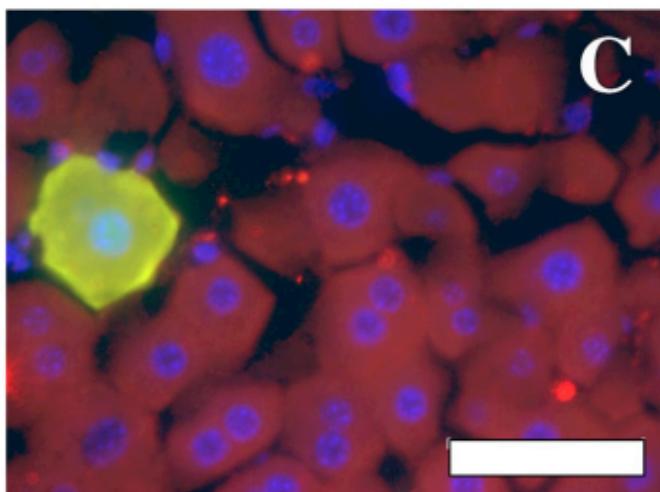
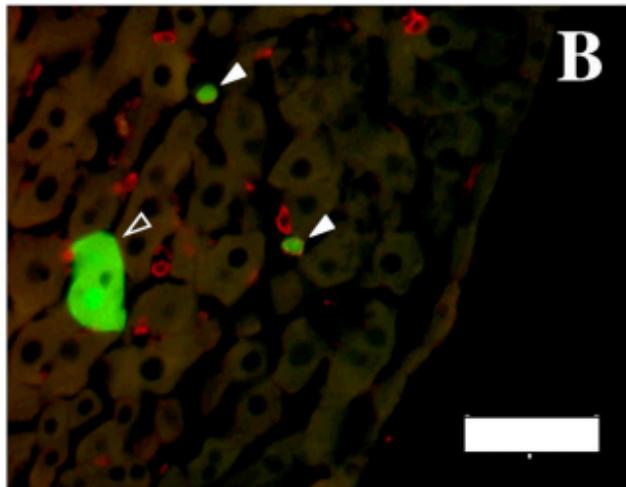
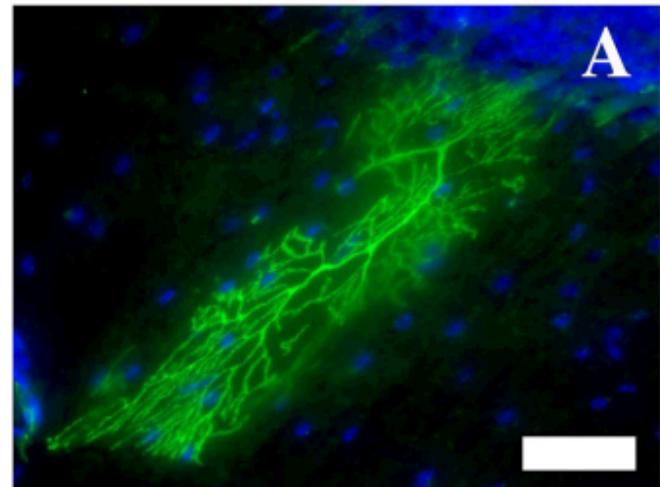


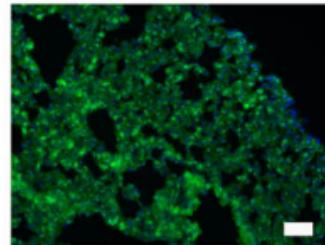
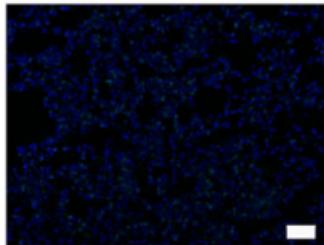
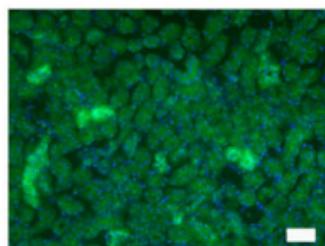
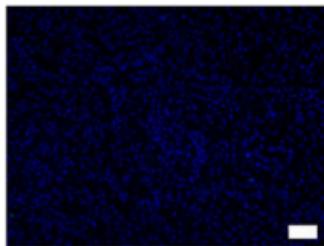
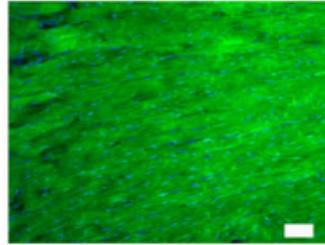
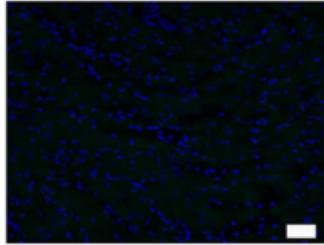
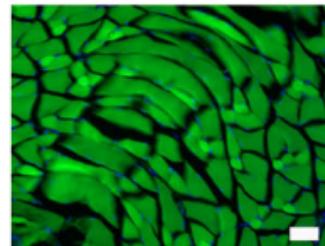
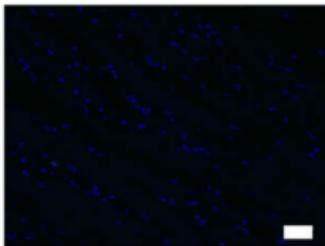
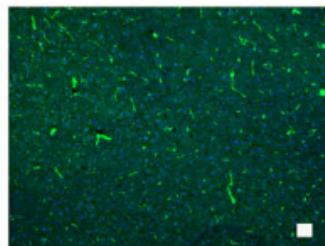
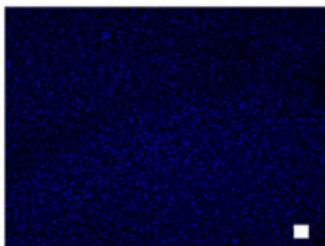
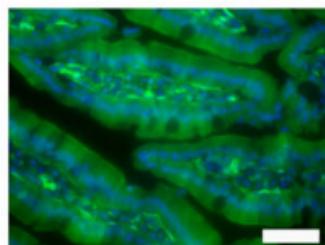
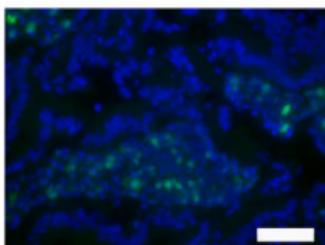
**Lung**

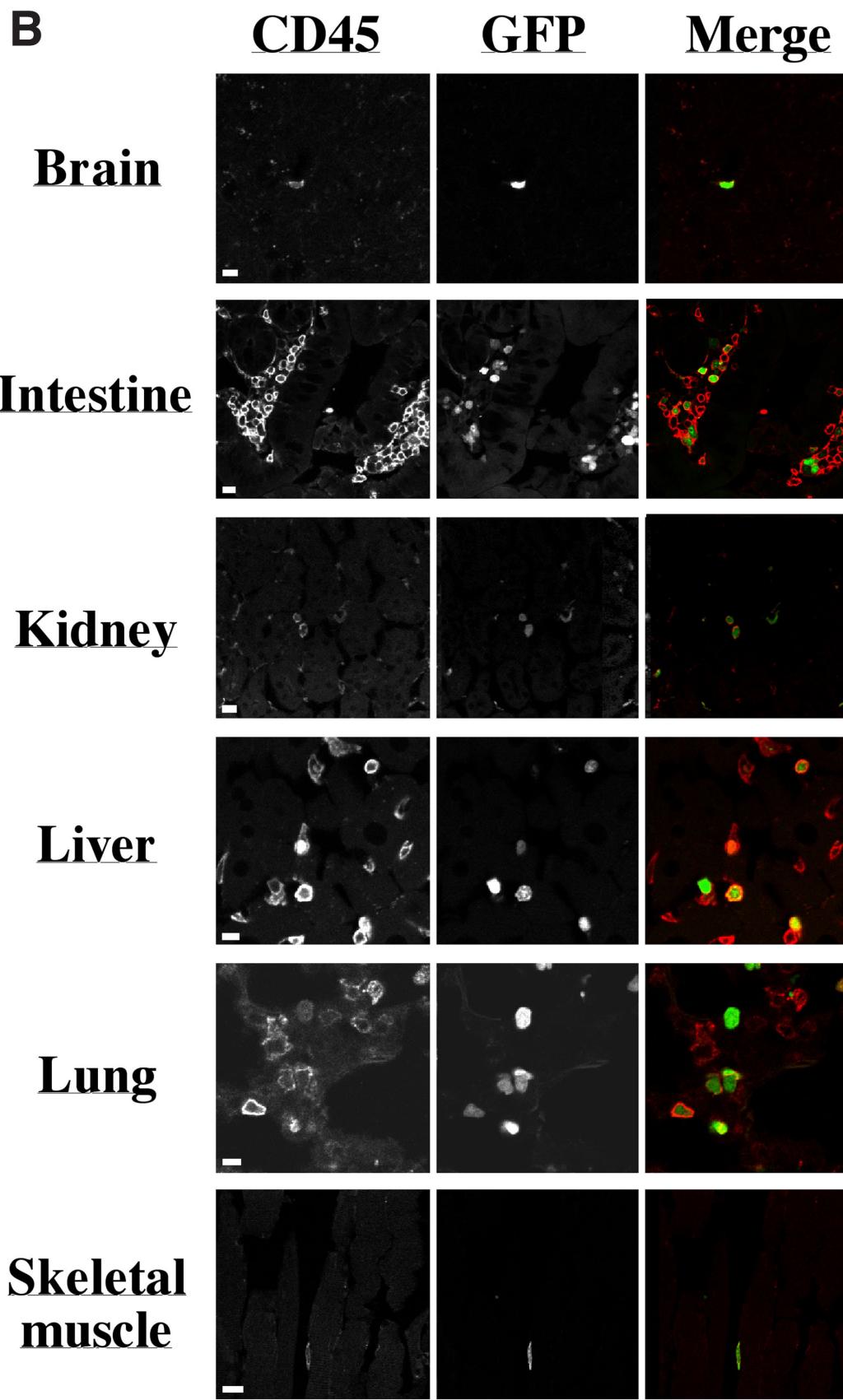


**Muscle**





**A**Control  
partnerGFP  
partnerLungKidneyCardiac  
muscleSkeletal  
muscleBrainIntestine



**Frequency of reconstitution (reconstituted mice/total)**

<b><u>5 weeks</u></b>	<b><u>14 weeks</u></b>
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<sup>+</sup> PB leukocytes)**

<b><u>5 weeks</u></b>	<b><u>14 weeks</u></b>
17.6% (Range: 0.12-77.6%)	20.2% (Range: 0.03-71.6%)

**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 post-transplant for the presence of GFP<sup>+</sup> donor-derived leukocytes. PB cells were stained for markers of the lymphoid (L) lineage (CD3<sup>+</sup>,B220<sup>+</sup>) versus myeloid (M) lineage (Mac-1<sup>+</sup>, Gr-1<sup>+</sup>) or separately for B cells (B, B220<sup>+</sup>), T cells (T, CD3<sup>+</sup>) and myeloid cells (M, Mac-1<sup>+</sup>, Gr-1<sup>+</sup>).

Tissue	# sections examined	~# cells examined	# GFP <sup>+</sup> non-hematopoietic 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	2355	0
Cardiac muscle	14	4346	0
Lung	12	23,000	0

**Table 2. Analysis of HSC-derived cells in single HSC transplanted mice.** Frozen sections of the indicated tissues from single GFP<sup>+</sup> HSC transplanted mice were analyzed for the presence of “transdifferentiated” cell types (18). The criteria for the identification of GFP<sup>+</sup> non-hematopoietic cells were as given in the text.

	<i><b>nonTg partner</b></i>			<i><b>GFP<sup>+</sup> partner</b></i>			<i><b>Average chimerism ± SD</b></i>
	<b>Pair 1</b>	<b>Pair 2</b>	<b>Pair 3</b>	<b>Pair 1</b>	<b>Pair 2</b>	<b>Pair 3</b>	
<b>Blood</b>	22.1	71.2	83.4	78.4	28.2	10.4	$49.0 \pm 32.2$
<b>BM</b>	4.69	73	19.2	81.3	14.4	7.82	$33.4 \pm 34.4$
<b>BM HSC</b>	24.9	69.4	10.5	27.2	11.4	2	$24.2 \pm 24.1$

**Table 3. Chimerism in blood and bone marrow of long-term parabionts.** Non-transgenic (nonTg) and GFP<sup>+</sup> 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<sup>+</sup> cells in the nonTg partner and %GFP<sup>-</sup> cells in the GFP<sup>+</sup> partner. Chimerism of BM HSC was determined following staining of BM cells for c-kit, Thy1.1, lineage, and Sca-1 (KTLS).

Tissue	# sections examined	~# cells examined	# partner-derived non-hematopoietic 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	3037	0
Cardiac muscle	10	2014	0
Lung	6	226,000	0

**Table 4. Analysis of partner-derived GFP<sup>+</sup> cross-engrafting cells in parabiotic mice.**

Frozen sections of the indicated tissues from non-transgenic partners of parabiotic pairs were analyzed for the presence of “transdifferentiated” cell types (18). The criteria for the identification of partner-derived GFP<sup>+</sup> non-hematopoietic cells were as given in the text.

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