Serum and organ iron content
Hepatic and splenic iron contents were determined by atomic absorption or colorimetric nonheme iron analysis as decribed.[1] Serum iron and transferrin saturation levels were determined using the Iron/UIBC kit from Thermo Electron (Melbourne, Australia) as described.[1]

Hematological measurements
Blood samples were obtained by retro-orbital puncture under anesthesia. CBCs were performed on an Advia 120 Hematology System (Bayer, Tarrytown, NY).

Flow cytometry
FACS analysis was performed as described.[1] In brief, 1×10^6 BM and spleen cells were washed and then incubated with either 0.1 ug of FITC-labeled anti-mouse CD71, APC-conjugated anti-mouse Ter119 and PE-conjugated anti-mouse CD44 antibodies (BD PharMingen, San Diego, CA) or isotype control antibodies in PBS-1% BSA at 4°C for 15 minutes. Flow-Jo software (Tree Star, Ashland, OR) was utilized for analysis.

Quantitative real-time PCR
Quantitave real-time PCR was performed as described.[1] We used previously described primers for *Hfe*,[1] *Hamp*,[1] *Bmp6*,[2] and *Id1*.[2]

Transduction of MEL cells
Aliquots (10^5 cells) of MEL cells were seeded in 6-well plates and serial dilutions of PHGW virus were added to the cells. Complete medium (RPMI, 10% fetal bovine serum, 100 IU/mL penicillin, 100 ug/mL streptomycin) was added to a final volume of 1 ml after which the cells were cultured overnight. The next day fresh complete medium was added and the cells were allowed to expand for at least 5 days prior to analysis. For fluorescent analysis 10^6 cells were incubated on ice for 15 minutes with 0.1 ug of PE-labeled anti–mouse CD71 (BD PharMingen) in PBS-1% BSA. Cells were washed twice with PBS-1% BSA and resuspended in 50 ul of PBS-1% BSA. Unfixed cells were analyzed immediately by fluorescence microscopy.

Table S1. Primers utilized for RT-PCR

	Forward primer	Reverse primer
Hfe	TACAATCATGAGAGTCGCCGTGCT	AGGTGATCTTGCCCGTCATAACCA
Tfr-2	TCCCGTCCTTCAATCAAACCCAGT	TTCGAGGTCTGAACCCATTGCTGA
Fpn1	ATGTGGCACTTTGCAGTGTCTGTG	AGGTGAAGGCCACAGTTCCCATTA
β-actin	GTGGGCCGCTCTAGGCACCA	CGGTTGGCCTTAGGGTTCA

Table S2. Hematological and iron parameters in bone marrow transplanted mice

	Hb (g/dL)	RBC ($\times 10^6$/ul)	Hct (%)	MCV (fL)	MCH (pg)	Retic ($\times 10^9$/L)	LIC (ug/mg dry)
wt→wt	14.3 ±0.2	10.2 ±0.1	46.6 ±0.7	45.7 ±0.2	14.0 ±0.1	276 ±12	0.29 ±0.02
***Hfe*-KO →wt**	14.0 ±0.2	9.7 ±0.1*	45.8 ±0.7	46.5 ±0.2	14.5 ±0.1*	263 ±9	0.31 ±0.07
wt→*Hfe*-KO	15.6 ±0.2**	9.7 ±0.1*	46.9 ±0.6	48.6 ±0.3***	16.1 ±0.1***	321 ±10	1.05 ±0.05**
***Hfe*-KO→*Hfe*-KO**	15.8 ±0.1**	9.9 ±0.1	47.7 ±0.2	47.9 ±0.1***	15.8 ±0.1***	301 ±13	1.10 ±0.26**

Values of hemoglobin (Hb), red blood cell count (RBC), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocyte count (Retic), and liver iron concentration (LIC) are shown as the means ± SEMs of 5–20 animals. $^{*}p \leq 0.05$, *and* $^{***}p \leq 0.001$ relative to wt→wt.

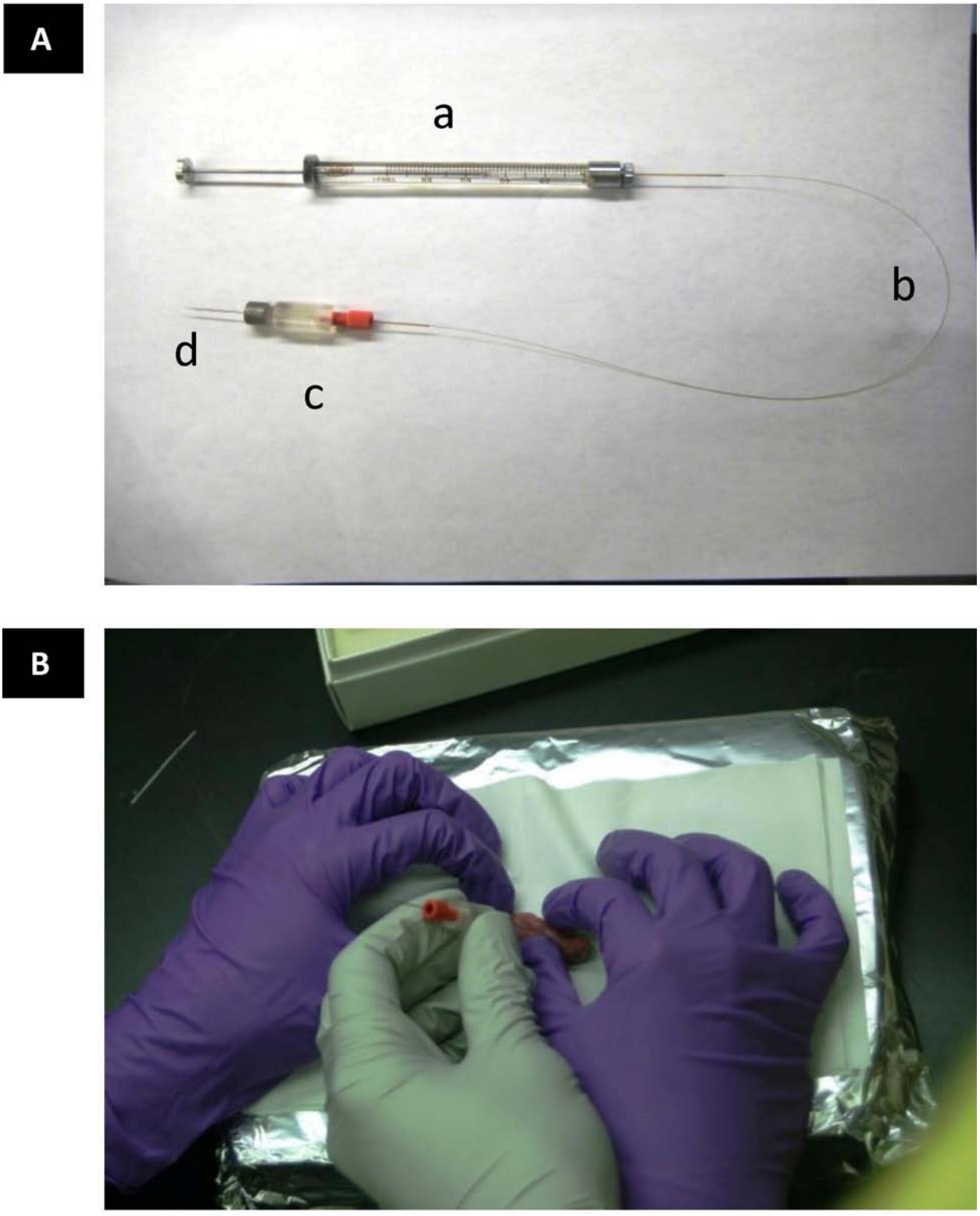

Figure S1. Hepatic lentiviral injection using a microinjector. (S1A) Micro injector consisting of four different pieces: (a) 100-µl nanofil syringe, (b) SilFlex tubing, (c) connector and (d) 35-gauge beveled needle. (S1B) A photograph depicting lentiviral injection into the liver of 3-day-old pups. The mouse is held in a supine position while lentivirus is gently injected into the liver using a micro injector.

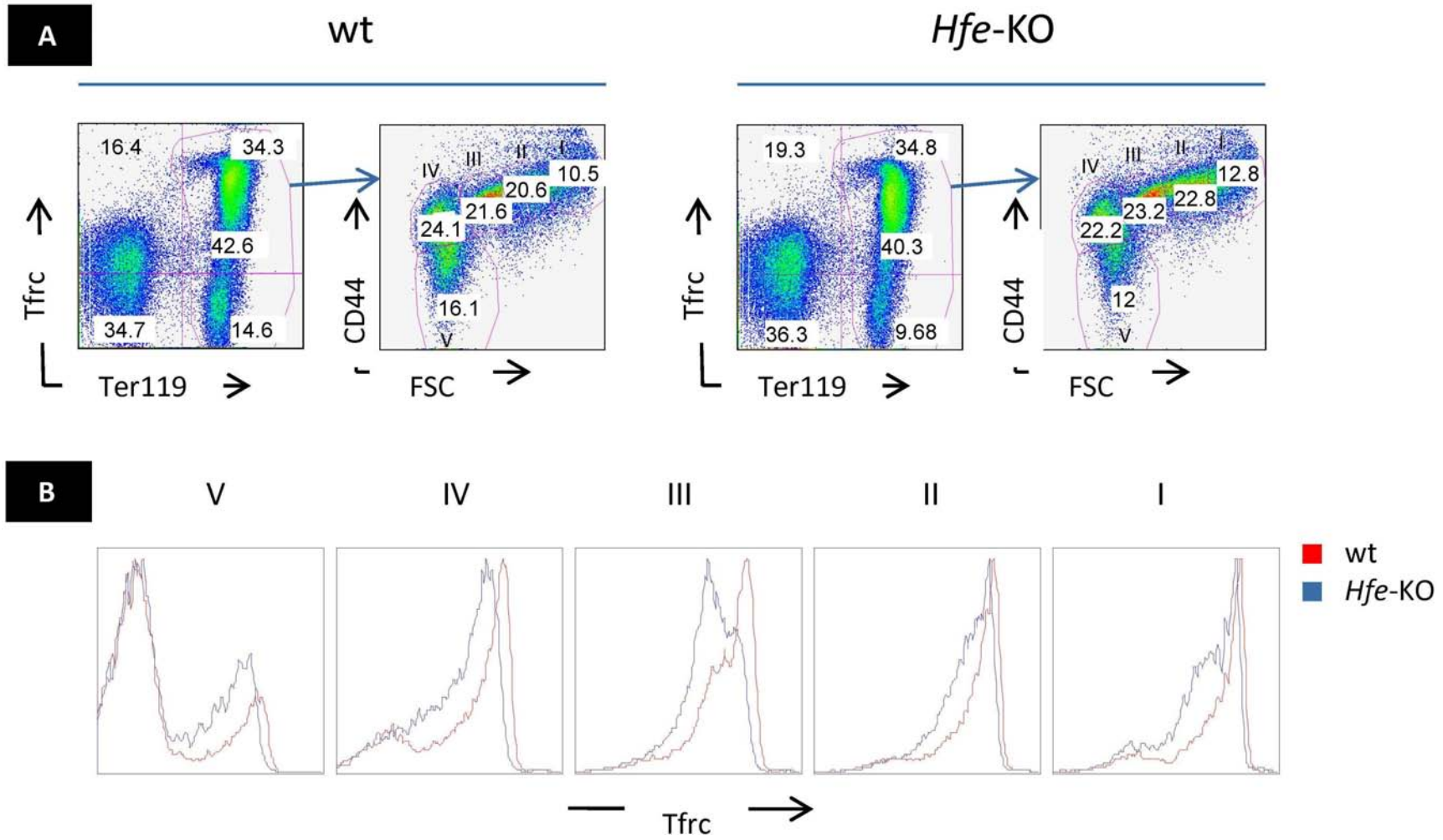

Figure S2. Tfrc is downregulated in membrane of *Hfe*-KO erythroid cells. (S2A) FACS profile of bone marrow cells co-stained with CD71 (Tfrc), Ter119 and CD44. For each genotype the left panel represents the classical CD71/Ter119 profiles allowing for separation of more immature (upper right quadrant) and more mature (lower right quadrant) cells. The right panel represents CD44 vs FSC (forward scatter) on the erythroid gate established in the CD71/Ter119 profile. Populations I, II, III, IV and V represent distinct erythroid populations progressively more mature as described by Chen K et al.[3] (S2B) Shows histograms of CD71 expression in populations I–V gated on the CD44/FSC profile. There is a clear shift to the left in histogram of *Hfe*-KO compared to wt cells in all populations gated showing decreased expression of CD71 in the membranes of *Hfe*-KO erythroid cells. This is a representative example of several independent experiments showing consistently similar results. The animals used in this particular example were 12 months old. Similar results were observed at different ages.

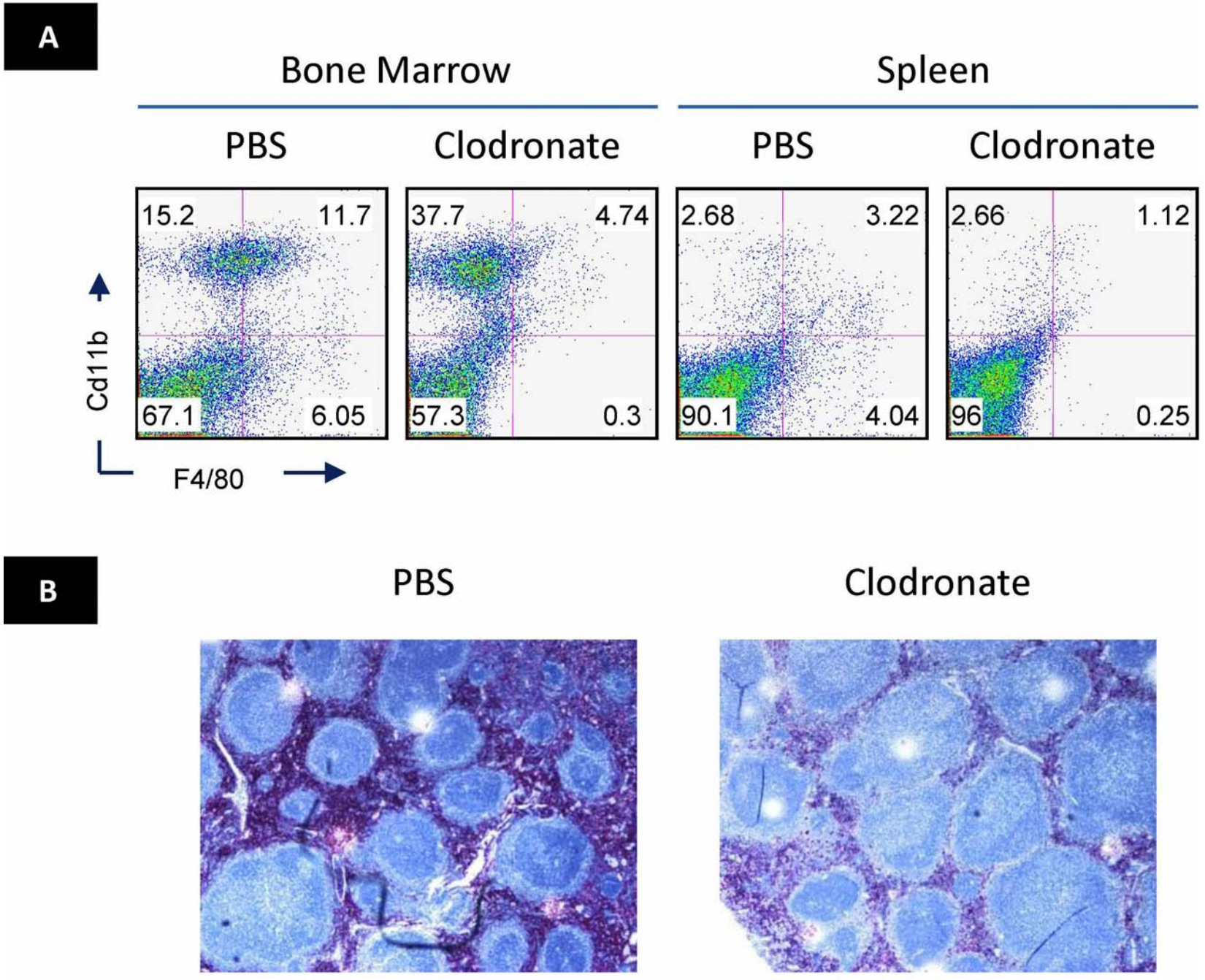
A
Bone Marrow
Spleen
PBS
Clodronate
PBS
Clodronate
15.2
11.7
67.1
6.05
37.7
4.74
57.3
0.3
2.68
3.22
90.1
4.04
2.66
1.12
96
0.25
Cd11b
F4/80
B
PBS
Clodronate

Figure S3. Effectiveness of clodronate-containing liposomes in causing macrophage depletion in bone marrow and spleen. (S3A) Splenic and bone marrow cells were stained with the myeloid/macropahge marker CD11b and the pan macrophage marker F4/80 allowing us to detect different macrophage populations. In both bone marrow and spleen it is clear that the percentages of F4/80 positive cells is markedly decreased in clodronate- compared to PBS-treated mice. (S3B) Immunohistochemistry of splenic sections with F4/80 showing marked reduction of staining (Red) in clodronate-treated mice compared to controls.

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