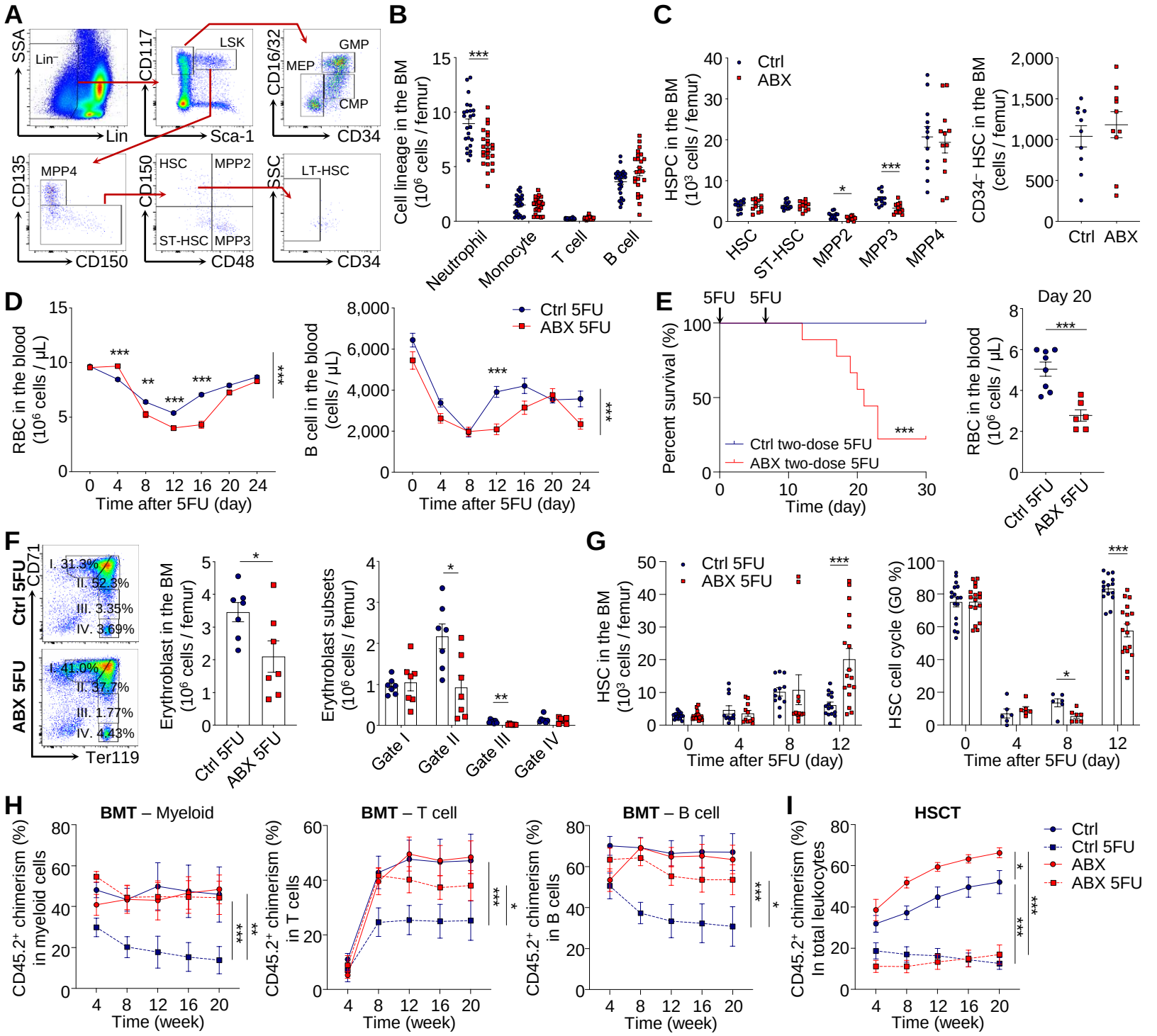


Figure S1. Microbiota depletion leads to impaired regeneration following 5FU challenge. *Related to Figure 1*

(A–C) Cell lineages, MPPs and HSCs in control and ABX-treated mice under steady state (n = 10–24). *See also Figure 1E and Figure S1H–I for long-term reconstitution following BMT and HSCT.*

(D) RBCs and B cells in the blood of control and ABX-treated mice after 5FU challenge (n = 6–27).

(E) Survival and RBCs in control and ABX-treated mice following two-dose 5FU challenge (n = 6–11).

(F) Erythroblast populations in control and ABX-treated mice at day 12 after 5FU challenge (n = 7).

(G) Time-course analyses of HSCs and HSC cell cyclinng in the BM of control and ABX-treated mice after 5FU challenge (n = 6–17).

(H–I) Multi-lineage reconstitution following BMT in control and ABX-treated mice under steady state or at day 12 after 5FU challenge (n = 5–7); HSCT analysis of sorted HSCs from the same conditions (n = 7–10).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars, mean ± SEM.

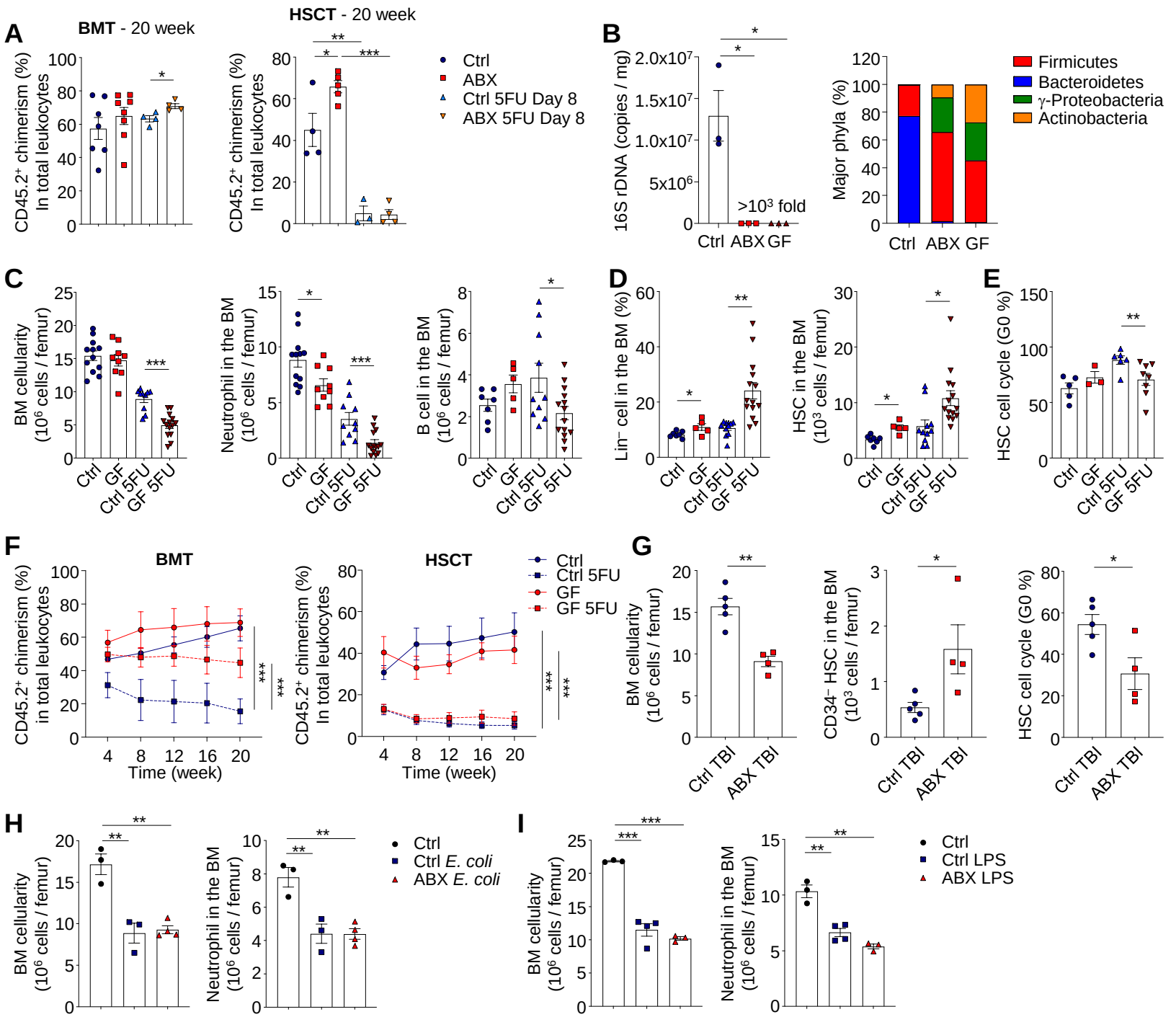


Figure S2. The microbiota regulates HSC response in stress conditions. ***Related to Figure 1 and 2***

(A) BMT and HSCT analyses of control and ABX-treated mice under steady state or at day 8 after 5FU challenge (n = 3–8).

(B) Total 16S rDNA copy numbers and the percentages of major phyla constituents of the microbiota in control, ABX-treated and GF animals at day 12 following 5FU challenge (n = 3).

(C–E) Total BM cellularity, neutrophils and B cells, Lin⁻ cells, HSCs, and HSC cell cycling in control and GF mice under steady state or at day 12 after 5FU challenge (n = 3–14).

(F) BMT and HSCT analyses of control and GF mice under steady state or at day 12 after 5FU challenge (n = 3–10).

(G) BM Cellularity, CD34⁻ LT-HSCs and HSC cell cycling in control and ABX-treated mice at day 24 after sublethal TBI (n = 4–5).

(H–I) Total cellularity and neutrophils in the BM of control and ABX-treated mice with or without *E. coli* infection or LPS challenge (n = 3–4).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars, mean ± SEM.

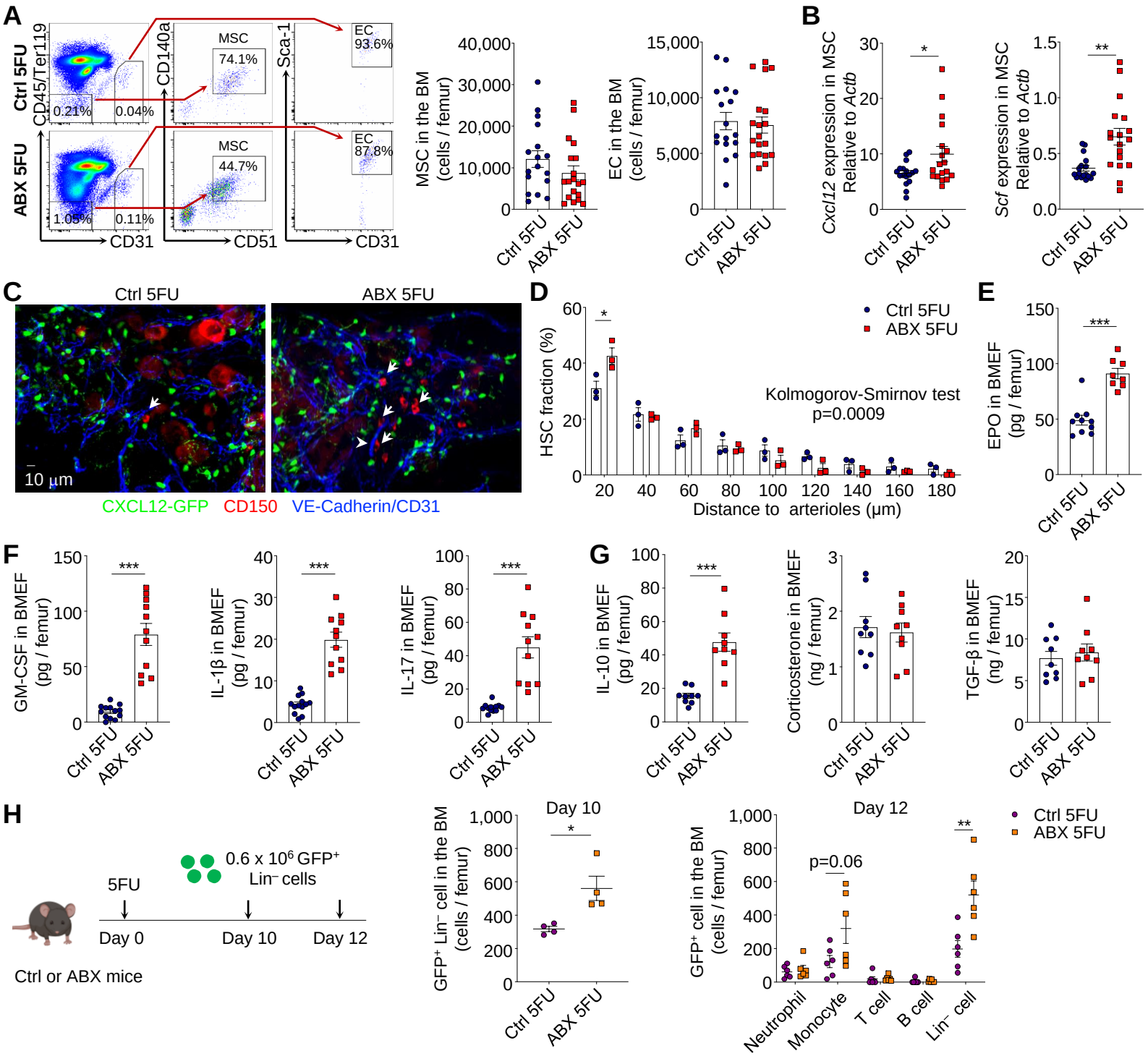


Figure S3. Microbiota depletion alters the BM microenvironment in regenerative condition. *Related to Figure 1*

(A–B) MSC and EC numbers, and the expression levels of niche factors in MSCs from control and ABX-treated mice at day 12 after 5FU challenge (n = 17–19).

(C–D) HSC distribution assessed by immunofluorescence in control and ABX-treated mice at day 12 after 5FU challenge (n = 3). Arrows point at HSCs identified by CD150⁺ Lin⁻ CD48⁻ staining; arrowhead points at arterioles. Scale bar, 10 µm.

(E–G) Erythropoietic, pro-inflammatory and anti-inflammatory cytokine levels in bone marrow extracellular fluid (BMEF) at day 12 after 5FU challenge (n = 8–13).

(H) Adoptive transfer of GFP⁺ Lin⁻ cells to evaluate progenitor homing and differentiation in control and ABX-treated mice. Mice analyzed on the same day for homing and two days later for differentiation (n = 4–6).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars, mean ± SEM.

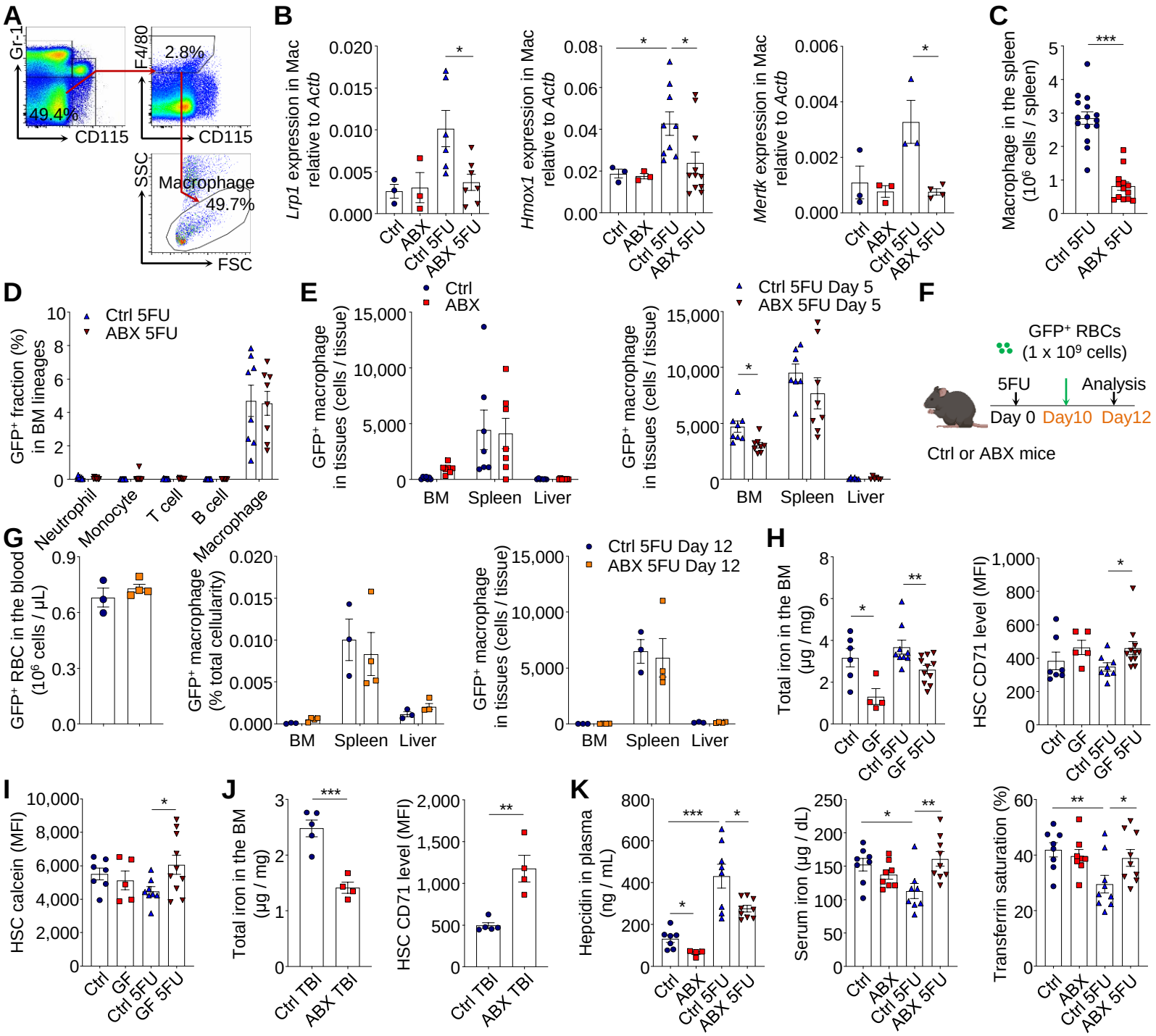


Figure S4. The microbiota regulates macrophage-mediated erythrophagocytosis and local iron availability during regeneration. *Related to Figure 3*

(A–B) Macrophage gating and the expression levels of phagocytosis and RBC degradation-related genes in BM macrophages of control and ABX-treated mice under steady state or at day 12 after 5FU challenge (n = 3–11). *Lrp1*, low density lipoprotein receptor-related protein 1; *Hmox1*, heme oxygenase 1; *Mertk*, proto-oncogene tyrosine-protein kinase MER. *See also Figure S6E for gene expression in the early stages.*

(C) Splenic macrophages in control and ABX-treated mice at day 12 after 5FU challenge (n = 13–15).

(D–G) GFP$^+$ fraction in BM cell lineages, and GFP$^+$ macrophages in different tissues in control and ABX-treated mice under steady state or in the early stages of regeneration (day 3–5; n = 6–8); GFP$^+$ RBC clearance and phagocytosis in the late stages of regeneration (day 10–12 ; n = 3–4).

(H–J) BM Iron levels, HSC CD71 expression and calcein fluorescence in control and GF mice under steady state or at day 12 after 5FU challenge (n = 4–11); in control and ABX-treated mice at day 24 after sublethal irradiation (n = 4–5). *See also Figure S6G calcein analysis in ABX-treated mice.*

(K) Hepcidin levels, serum iron levels and transferrin saturation in control and ABX-treated mice under steady state or at day 12 after 5FU challenge (n = 4–9).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars, mean ± SEM.

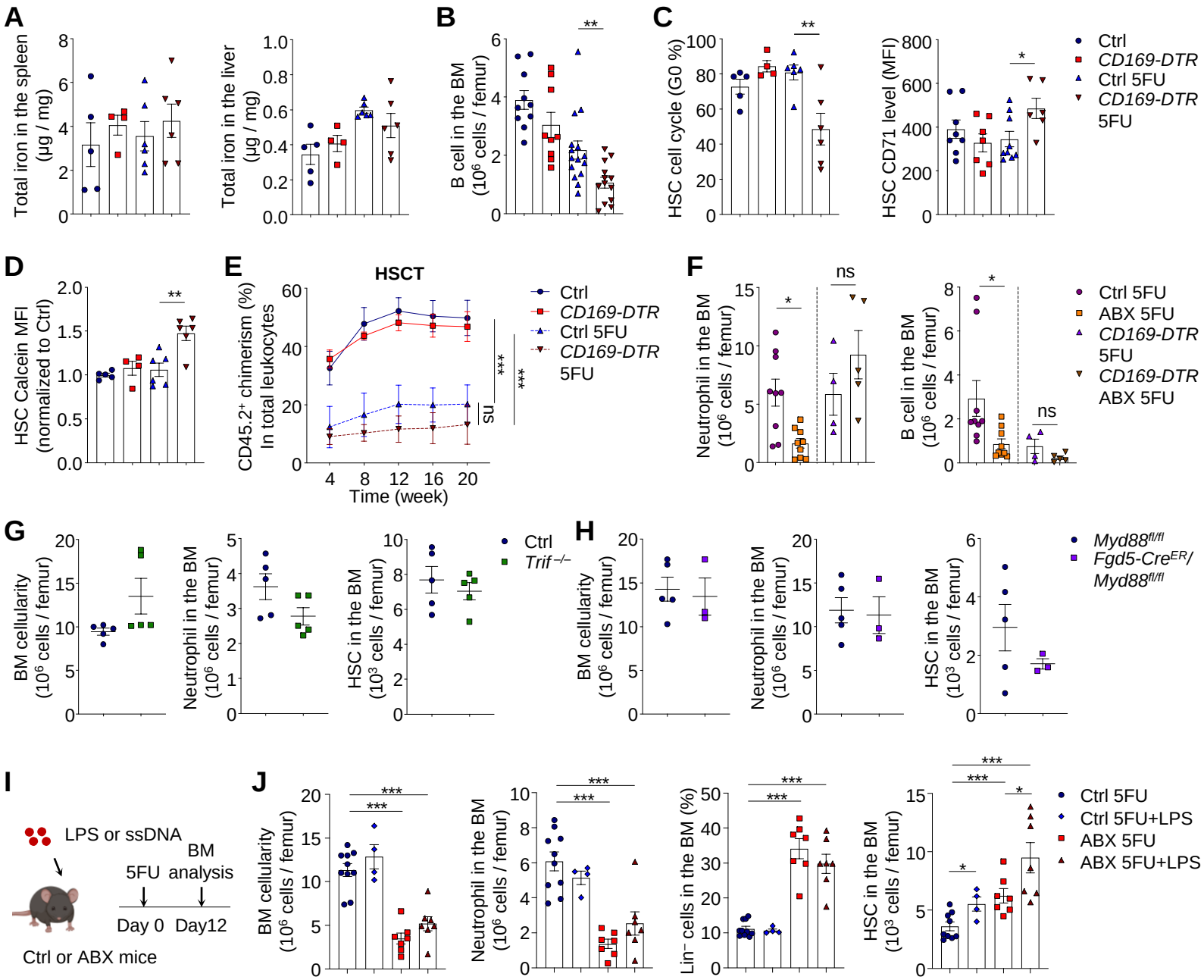


Figure S5. BM macrophages mediate the crosstalk between the microbiota and HSCs. *Related to Figure 4 and Figure 5*

(A) Spleen and liver iron levels in control and *CD169-DTR* mice under steady state or at day 12 after 5FU challenge (n = 4–6).

(B–E) B cells, HSC cell cycling, CD71 expression, calcein fluorescence and HSCT analysis in control and *CD169-DTR* mice under steady state or at day 12 after 5FU challenge (n = 4–15).

(F) Neutrophils and B cells in control or *CD169-DTR* mice with or without ABX treatment at day 12 after 5FU challenge (n = 4–9).

(G–H) BM cellularity, neutrophils and HSCs at day 12 after 5FU challenge in *Trif*$^{-/-}$ mice (n = 5); and in *Fgd5-Cre*^{ER}/ *Myd88*$^{fl/fl}$ mice (n = 3–5).

(I–J) BM cellularity, neutrophils, Lin^{-} cells, and HSCs in control and ABX-treated mice with or without LPS gavage, at day 12 after 5FU challenge (n = 4–10).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant. Error bars, mean ± SEM.

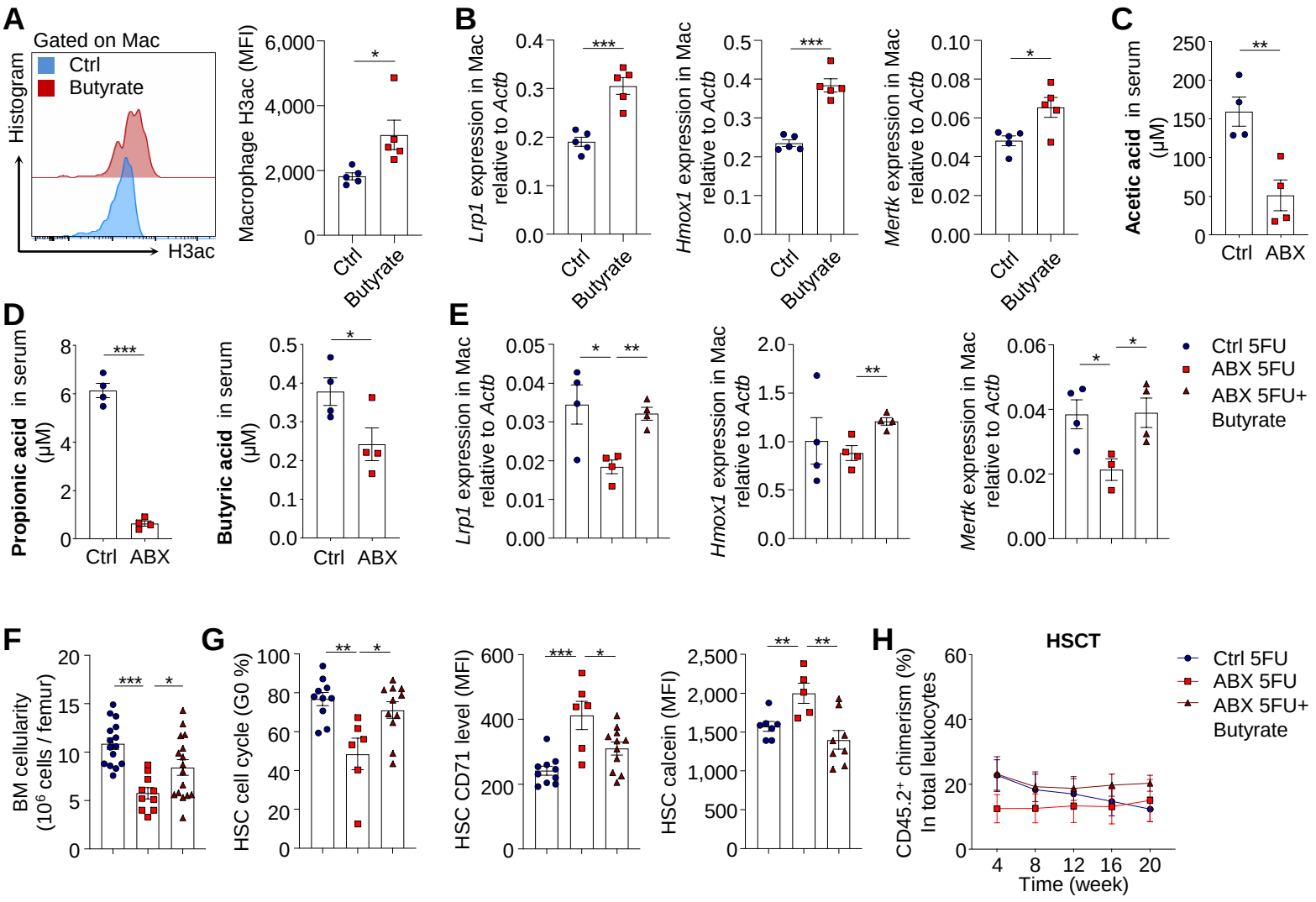


Figure S6. The microbial metabolite butyrate orchestrates macrophage function, iron availability and HSC response in the BM during regeneration. *Related to Figure 5*

(A–B) Histone H3 acetylation (H3ac) levels and expression levels of *Lrp1*, *Hmox1* and *Mertk* in BM-derived macrophages cultured with or without butyrate (n = 5).

(C–D) Quantification of serum levels of major SCFA species in control or ABX-treated mice (n = 4).

(E) Expression levels of *Lrp1*, *Hmox1* and *Mertk* in BM macrophages of control, ABX-treated and ABX-treated mice supplemented with butyrate, in the early phase of regeneration (day 3–5; n = 3–4) .

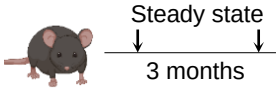
(F–G) BM cellularity, HSC cell cycling, CD71 expression and calcein fluorescence in control, ABX-treated and ABX-treated mice supplemented with butyrate, at day 12 after 5FU treatment (n = 5–16).

(H) HSCT analysis of control, ABX-treated or ABX-treated mice supplemented with butyrate, at day 12 after 5FU challenge (n = 6–7).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars, mean ± SEM.

A

Iron-adjusted diet:
Ctrl: 185ppm
Iron Low: 30ppm
Iron Deficient: 3ppm

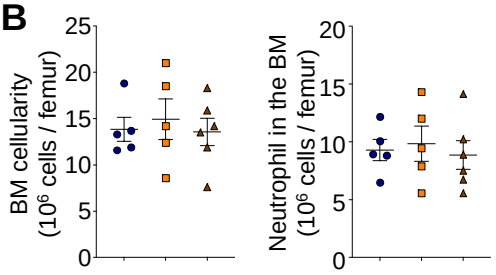


C57BL/6 mice

	RBC (10^6 cells/μL)	Hemoglobin (g/dL)	MCV (fL)	Neutrophil (cells/μL)
Ctrl	9.8±0.2	14.2±0.3	52.7±0.6	741±142
Iron Low	9.9±0.2	13.2±0.4	51.2±0.5	573±105
Iron Def	10.8±0.4 *	12.1±0.3 ***	45.6±1.7 ***	378±34 *

MCV, Mean corpuscular volume.
Data are presented as Mean±SEM.

B



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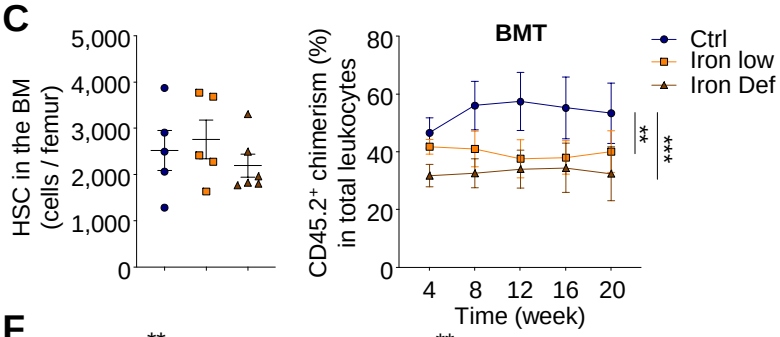

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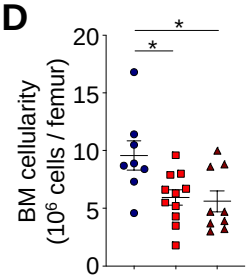

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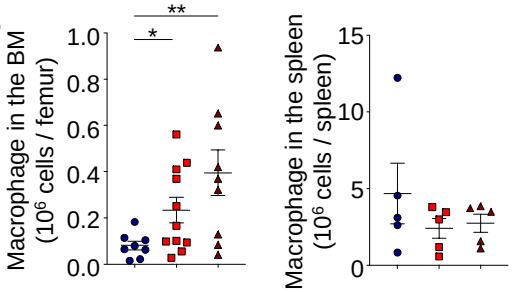

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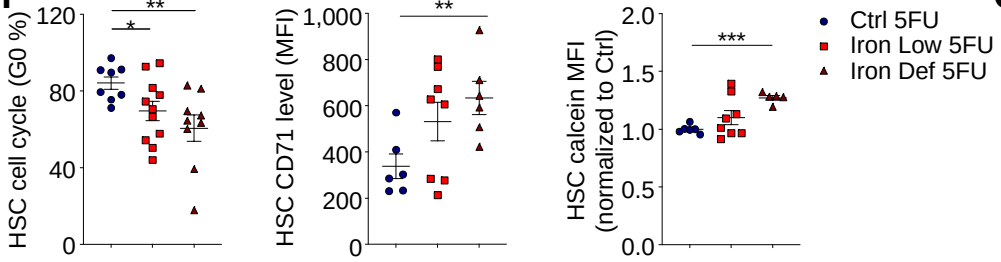

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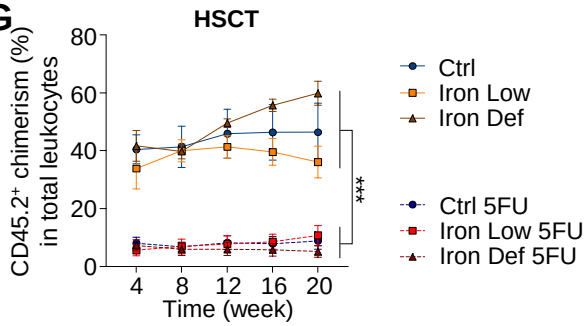

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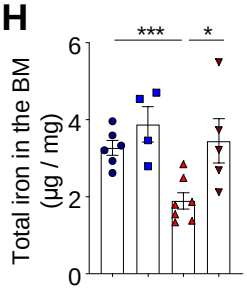

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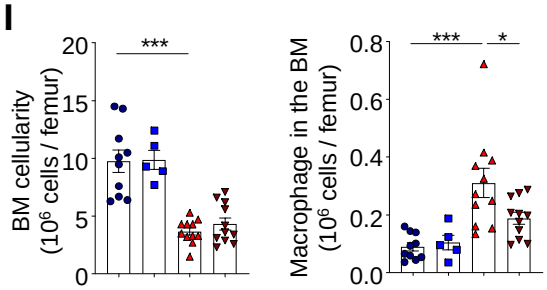

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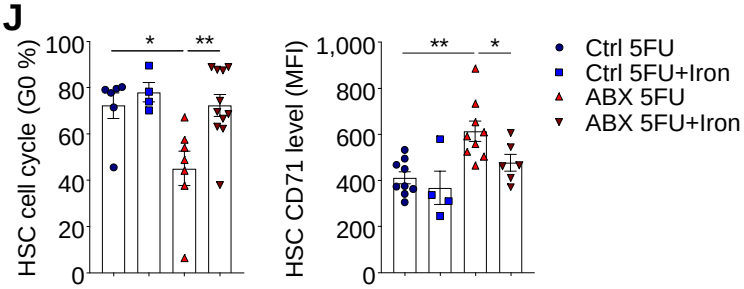

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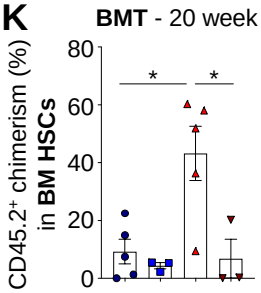

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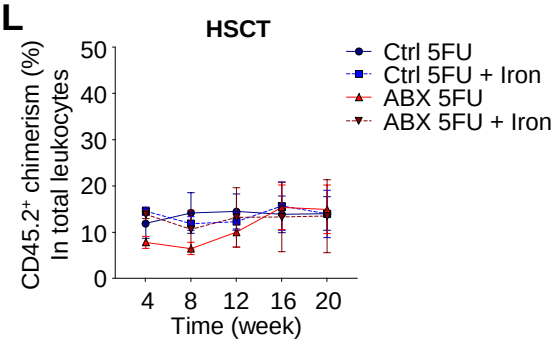

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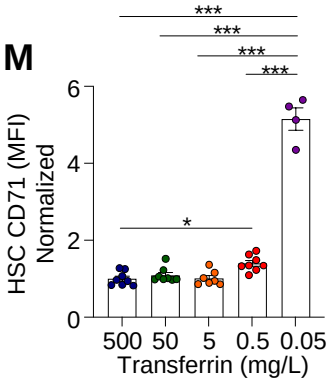

Figure S7. Manipulations of iron levels in steady-state, regenerative and culture conditions. ***Related to Figure 6 and Figure 7***

(A–C) Blood parameters, BM cellularity, neutrophils, HSCs, and long-term reconstitution following BMT under steady state in mice fed with normal, iron-low or iron-deficient food (n = 5–9).

(D–F) BM cellularity, BM and splenic macrophages, HSC cell cycling, CD71 expression and calcein fluorescence in mice fed with normal, iron-low or iron-deficient food, at day 12 after 5FU challenge (n= 5–11).

(G) HSCT analysis of mice fed with normal, iron-low or iron-deficient food under steady state or at day 12 after 5FU challenge (n= 3–9).

(H–J) BM iron levels, total cellularity, macrophages, HSC cell cycling and CD71 expression at day 12 after 5FU challenge in control or ABX-treated mice injected with PBS or iron dextran (n = 4–11).

(K) Percentages of $CD45.2^{+}$ donor cells in recipient BM HSCs 20 weeks post BMT (n= 3–5) .

(L) HSCT analysis of control or ABX-treated mice with or without iron supplementation, at day 12 after 5FU challenge (n= 3–5).

(M) CD71 expression levels of HSCs cultured in the PVA-based serum-free system with defined transferrin levels (n = 4–8).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars, mean ± SEM.

Table S1. Primers for real-time qPCR. ***Related to Figure S4B and S6E***

Gene Symbol (Mouse)	**Forward**	**Reverse**
Actb	GCTTCTTTGCAGCTCCTTCGT	ATCGTCATCCATGGCGAACT
Lrp1	ACTATGGATGCCCCTAAAACTTG	GCAATCTCTTTCACCGTCACA
Hmox1	GCCACCAAGGAGGTACACAT	GCTTGTTGCGCTCTATCTCC
Mertk	GATTCTGGCCAGCACAACAGA	GAGATATCCGGTAGCCCACCA

Table S2. Primers for 16S rDNA analysis. ***Related to Figure S2B***

Bacterial Phyla	**Forward**	**Reverse**
Pan-bacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Bacteroidetes	CRAACAGGATTAGATACCCT	GGTAAGGTTCCTCGCGTAT
Firmicutes	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC
γ-Proteobacteria	TCGTCAGCTCGTGTYGTGA	CGTAAGGGCCATGATG
Actinobacteria	TACGGCCGCAAGGCTA	TCRTCCCCACCTTCCTCCG