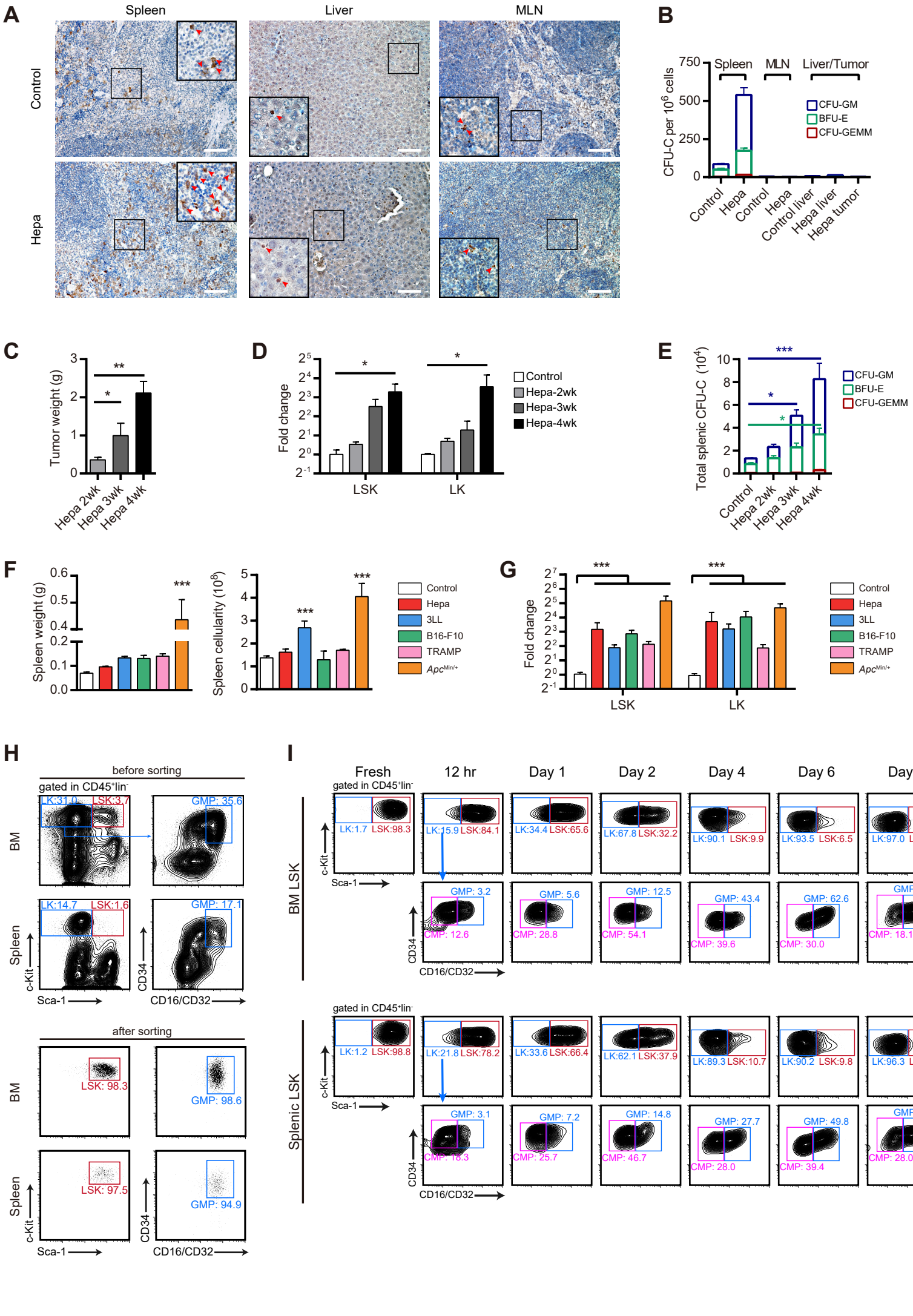
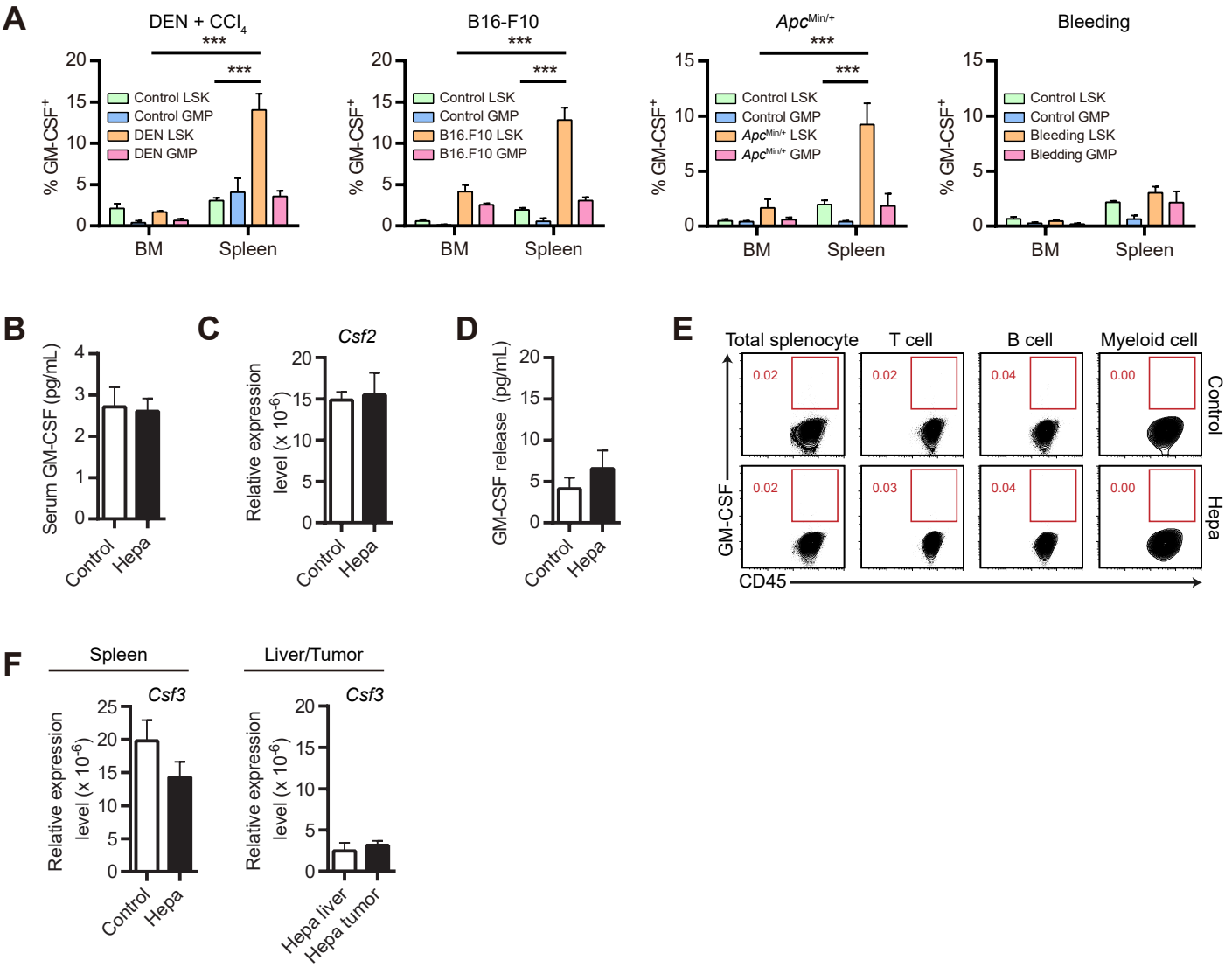
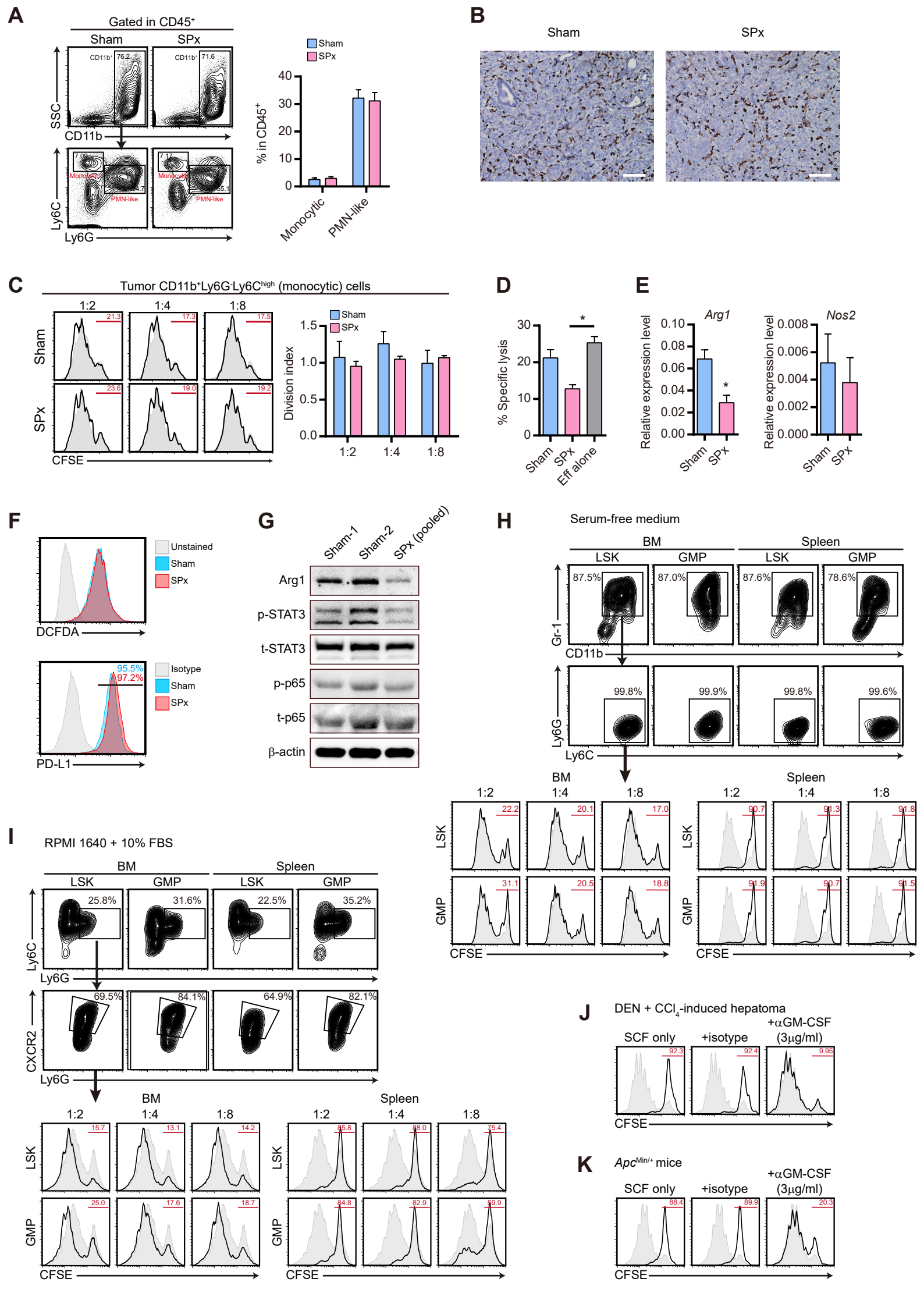

A
Spleen
Liver
MLN
Control
Hepa
B
CFU-C per 10^6 cells
Spleen
MLN
Liver/Tumor
CFU-GM
BFU-E
CFU-GEMM
Control
Hepa
Control
Hepa
Control liver
Hepa liver
Hepa tumor
C
Tumor weight (g)
Hepa 2wk
Hepa 3wk
Hepa 4wk
D
Fold change
Control
Hepa-2wk
Hepa-3wk
Hepa-4wk
LSK
LK
E
Total splenic CFU-C (10^4)
CFU-GM
BFU-E
CFU-GEMM
Control
Hepa 2wk
Hepa 3wk
Hepa 4wk
F
Spleen weight (g)
Spleen cellularity (10^8)
Control
Hepa
3LL
B16-F10
TRAMP
Apc Min/+
G
Fold change
LSK
LK
H
before sorting
gated in CD45+lin-
BM
Spleen
LK:31.0
LSK:3.7
GMP: 35.6
LK:14.7
LSK:1.6
GMP: 17.1
c-Kit
Sca-1
CD34
CD16/CD32
after sorting
LSK: 98.3
GMP: 98.6
LSK: 97.5
GMP: 94.9
I
Fresh
12 hr
Day 1
Day 2
Day 4
Day 6
Day 8
BM LSK
Splenic LSK
gated in CD45+lin-
LK:1.7 LSK:98.3
LK:15.9 LSK:84.1
LK:34.4 LSK:65.6
LK:67.8 LSK:32.2
LK:90.1 LSK:9.9
LK:93.5 LSK:6.5
LK:97.0 LSK:3.0
GMP: 3.2
CMP: 12.6
GMP: 5.6
CMP: 28.8
GMP: 12.5
CMP: 54.1
GMP: 43.4
CMP: 39.6
GMP: 62.6
CMP: 30.0
GMP: 77.8
CMP: 18.1
LK:1.2 LSK:98.8
LK:21.8 LSK:78.2
LK:33.6 LSK:66.4
LK:62.1 LSK:37.9
LK:89.3 LSK:10.7
LK:90.2 LSK:9.8
LK:96.3 LSK:3.7
GMP: 3.1
CMP: 18.3
GMP: 7.2
CMP: 25.7
GMP: 14.8
CMP: 46.7
GMP: 27.7
CMP: 28.0
GMP: 49.8
CMP: 39.4
GMP: 67.5
CMP: 28.0

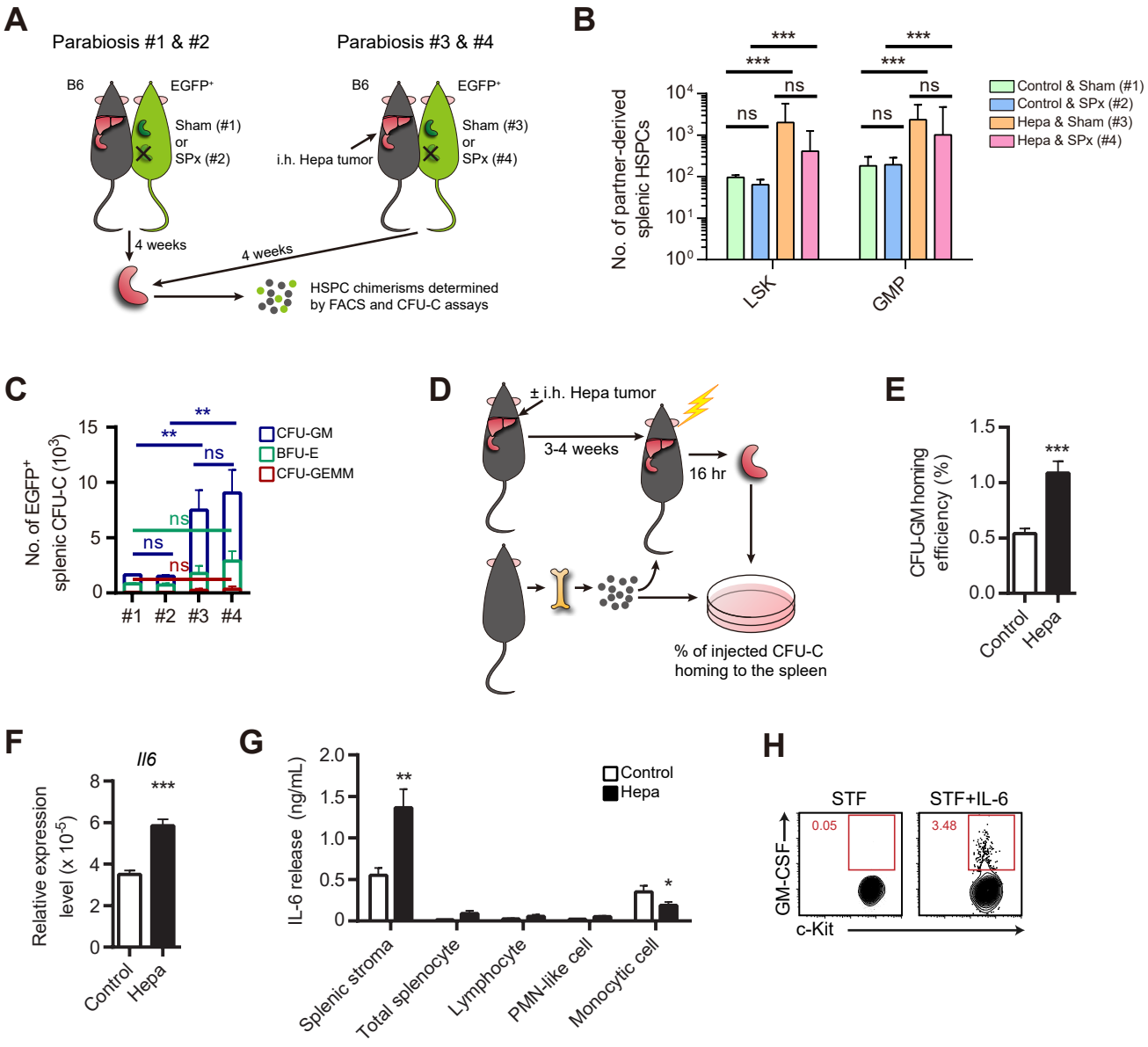
Supplemental Figure 1. Characteristics of cancer-induced splenic EMH. (**A**) Representative images of the histological detection of c-Kit^{+} progenitor cells in the spleens (left), non-cancerous liver lobes (middle) and mesenteric lymph nodes (MLN; right) of control or Hepa mice (N = 6–10 per group). Scale bar, 50 μm. (**B**) Numbers of CFU-C obtained with one million nucleated cells isolated from indicated tissues of control or Hepa mice (N = 6–10 per group; mean and s.e.m.). BFU-E: Burst-forming unit-erythroid; CFU-GM: Colony-forming unit-granulocyte/macrophage; CFU-GEMM: Colony-forming unit-granulocyte/erythrocyte/ macrophage/megakaryocyte. (**C**–**E**) Tumor weight (**C**), fold changes in splenic LSK and LK cell numbers (**D**), and CFU-C numbers in the spleens (**E**) of control or Hepa mice at different time points (N = 4 per group; mean and s.e.m.). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (one-way or two-way ANOVA followed by the Dunn's multiple comparison tests). (**F** and **G**) Spleen weight (**F**, left), total splenocyte numbers (**F**, right), and fold changes in splenic LSK and LK cell numbers (**G**) of control (N = 7), Hepa (N = 8), 3LL (N = 10), B16-F10 (N = 4), TRAMP (N = 2), and *Apc*$^{Min/+}$ (N = 8) mice. Result are shown as mean and s.e.m. ***$P < 0.001$ (Kruskal-Wallis test followed by the Dunn's multiple comparison test). (**H**) Gating strategy and the population purity after fluorescence-activated cell sorting (FACS). Numbers in plots indicate the proportions of gated cells. (**I**) Kinetics of BM and splenic LSK cells from Hepa mice to differentiate into CMPs and GMPs in vitro with SCF, TPO, and Flt3L in serum-free medium. Numbers in plots indicate the proportions of gated cells. Representative results from one of three independent experiments are shown.



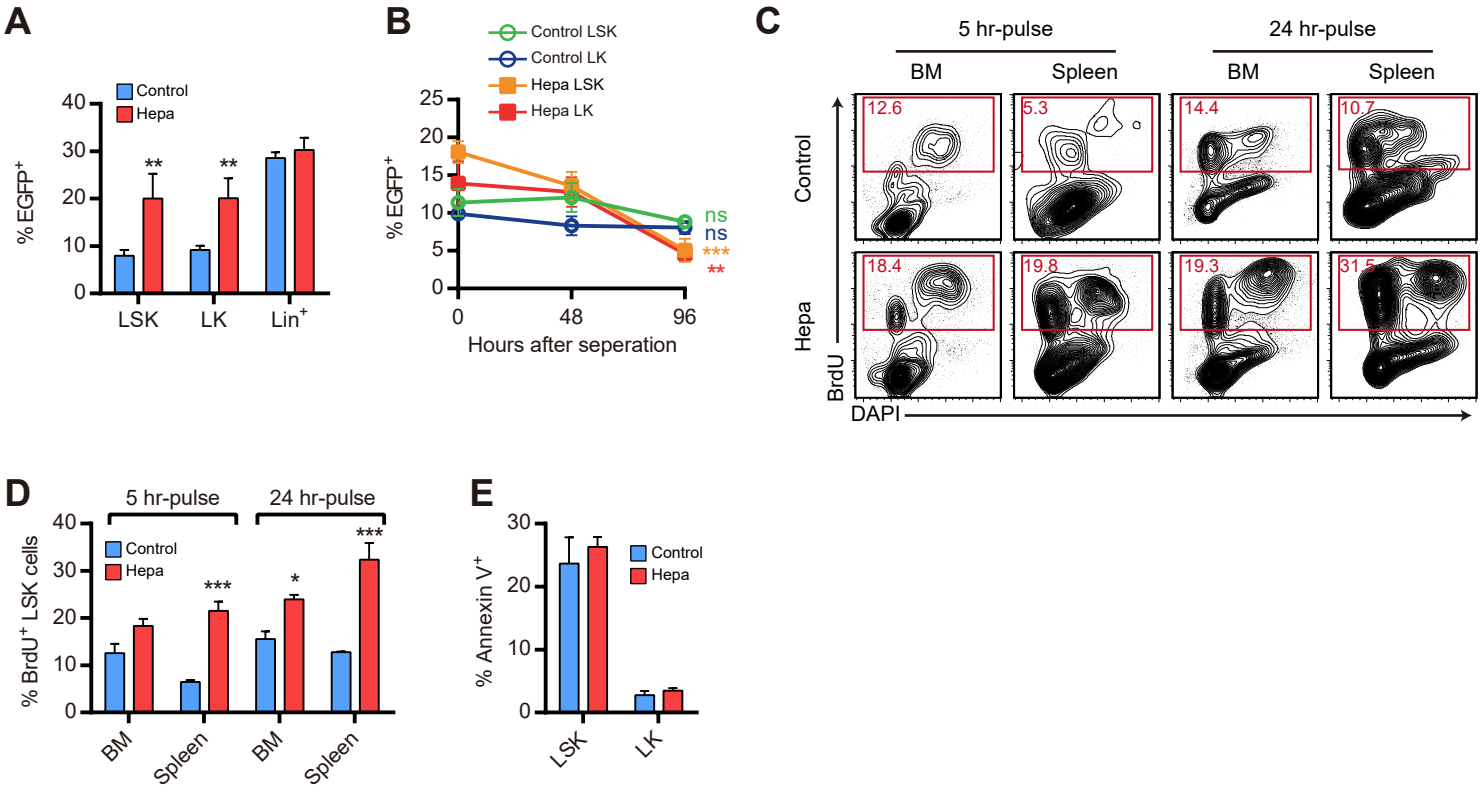
Supplemental Figure 2. GM-CSF-expressing LSK cells in the spleens of mice with different types of tumors. (**A**) The endogenous GM-CSF expression in LSK and GMP cells isolated from mice with hepatoma induced with DEN and CCl_4 (Control: $N = 3$; DEN: $N = 6$), mice bearing B16-F10 melanoma ($N = 4$ per group), 16-week-old $Apc^{Min/+}$ mice bearing intestinal neoplasia ($N = 3$ per group), or a model of blood loss-induced EMH ($N = 4$ per group). Data are showed as mean and s.e.m. $***P < 0.001$ (two-way ANOVA followed by Dunnett's test). (**B**) GM-CSF levels in the sera of control ($N = 5$) and Hepa ($N = 6$) mice, determined by ELISA. Data are showed as mean and s.e.m. $P > 0.05$ (Student's *t*-test). (**C**) The *Csf2* mRNA expression, relative to *Actb*, in the spleens of control and Hepa mice ($N = 7$ per group; mean and s.e.m.). $P > 0.05$ (Student's *t*-test). (**D**) GM-CSF release was measured in the supernatants of 48-hr cultures of splenic stromal cells from control or Hepa mice ($N = 5$–6 per group; mean and s.e.m.), by ELISA. $P > 0.05$ (Student's *t*-test). (**E**) The GM-CSF expression in indicated splenic cell populations from control or Hepa mice evaluated by flow cytometry ($N = 6$ per group; mean and s.e.m.). (**F**) The relative *Csf3* expression in the spleens of control and Hepa mice (left), or in the noncancerous liver and tumor tissues of Hepa mice. Values are normalized to *Actb* expression. Data are shown as mean and s.e.m. ($N = 4$–6 mice per group). $P > 0.05$ (Student's *t*-test).


A
Gated in CD45+
Sham
SPx
CD11b+
76.2
71.6
SSC
CD11b
7.06
7.17
Monocytic
PMN-like
Ly6C
Ly6G
Sham
SPx
% in CD45+
40
30
20
10
0
Monocytic
PMN-like
B
Sham
SPx
C
Tumor CD11b+Ly6G-Ly6Chigh (monocytic) cells
1:2
1:4
1:8
21.3
17.3
17.5
23.6
19.0
19.2
Sham
SPx
CFSE
Division index
1.5
1.0
0.5
0.0
D
% Specific lysis
30
20
10
0
Sham
SPx
Eff alone
E
Arg1
Nos2
Relative expression level
0.10
0.08
0.06
0.04
0.02
0.00
0.008
0.006
0.004
0.002
0.000
F
Unstained
Sham
SPx
DCFDA
95.5%
97.2%
Isotype
PD-L1
G
Sham-1
Sham-2
SPx (pooled)
Arg1
p-STAT3
t-STAT3
p-p65
t-p65
β-actin
H
Serum-free medium
BM
Spleen
LSK
GMP
87.5%
87.0%
87.6%
78.6%
Gr-1
CD11b
99.8%
99.9%
99.8%
99.6%
Ly6G
Ly6C
1:2
1:4
1:8
22.2
20.1
17.0
90.7
91.3
91.8
31.1
20.5
18.8
91.9
90.7
91.5
CFSE
I
RPMI 1640 + 10% FBS
25.8%
31.6%
22.5%
35.2%
69.5%
84.1%
64.9%
82.1%
CXCR2
15.7
13.1
14.2
25.0
17.6
18.7
85.8
88.0
75.4
84.8
82.9
89.9
J
DEN + CCl4-induced hepatoma
SCF only
+isotype
+αGM-CSF (3μg/ml)
92.3
92.4
9.95
K
Apc Min/+ mice
88.4
89.9
20.3

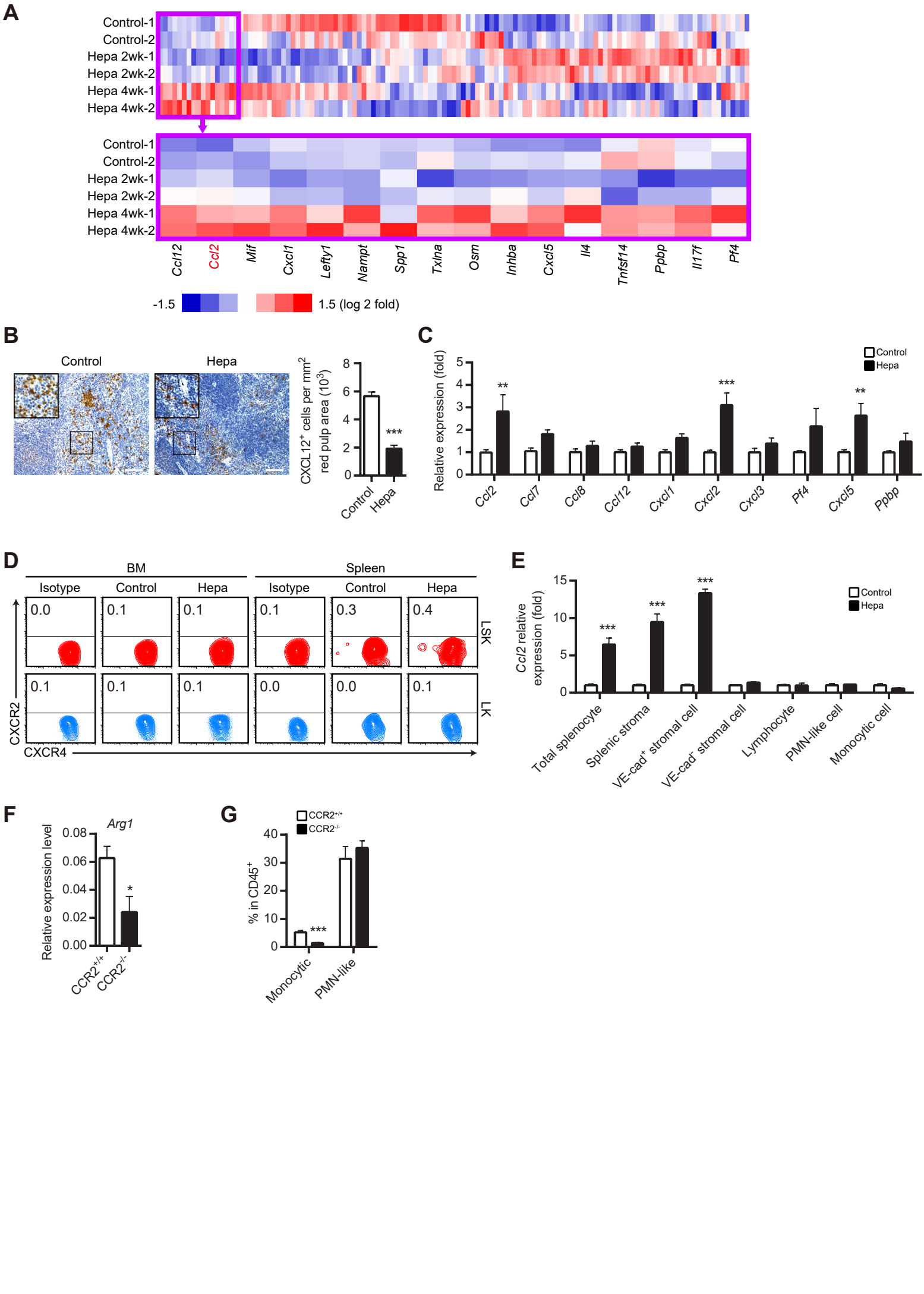
Supplemental Figure 3. Splenic HSPCs are committed to generating immunosuppressive myeloid cells. (**A**) Frequencies of tumor-infiltrating $CD11b^{+}Ly6G^{-}Ly6C^{high}$ monocytic and $CD11b^{+}Ly6G^{+}Ly6C^{low}$ PMN-like cells from Hepa mice subjected to a sham surgery (Sham) or splenectomy (SPx) were evaluated by flow cytometry. $P > 0.05$ (two-way ANOVA). (**B**) IHC staining for $CD11b^{+}$ in Hepa tumors. Scale bar, 50 μm. (**C** and **D**) The influence of tumor-infiltrating $CD11b^{+}Ly6G^{-}Ly6C^{high}$ monocytic myeloid cells on anti-CD3/28 stimulated T cell proliferation (**C**) and on the cytotoxic activity of $CD8^{+}$ T cells elicited by antigen-specific stimulation in vitro (**D**). *$P < 0.05$ (two-way ANOVA, corrected by Bonferroni's method). (**E**) The *Arg1* and *Nos2* mRNA expression in the tumor $CD11b^{+}Ly6G^{+}Ly6C^{low}$ PMN-like cells isolated from Hepa mice subjected to a sham surgery or splenectomy. Values are normalized to *Actb* expression. *$P < 0.05$ (Student's *t*-test). (**F**) Flow cytometric analysis of ROS and PD-L1 expression in the tumor-infiltrating $CD11b^{+}Ly6G^{+}Ly6C^{low}$ PMN-like cells. To determine the production of ROS, cells were loaded with DCFDA and the fluorescence intensity of DCF was measured. (**G**) Immunoblot for Arg1 expression, STAT3 and NF-κB p65 activation in tumor-infiltrating $CD11b^{+}Ly6G^{+}Ly6C^{low}$ PMN-like cells isolated from Hepa mice. p: phosphorylated; t: total. (**H** and **I**) BM and splenic LSK and GMP cells were cultured in serum-free medium (**H**) or RPMI 1640 complete medium (**I**), and induced with GM-CSF for 4 days. $Gr\text{-}1^{+}$ descendants (**H**) or $Ly6G^{+}Ly6C^{low}CXCR2^{+}$ descendants (**I**) were isolated and tested for their suppressive activity on anti-CD3/28 stimulated T cell proliferation at indicated ratios. (**J** and **K**) LSK cells were isolated from the spleens of mice with hepatoma induced with DEN and CCl_4 (**J**), or 16-week-old $Apc^{Min/+}$ mice bearing intestinal neoplasia (**K**). The LSK cells were then cultured in serum-free medium with SCF for four days. The $Gr\text{-}1^{+}$ myeloid descendants were isolated and tested for their suppressive function on anti-CD3/28 stimulated T cell proliferation at the ratio of 1:4. Solid lines in plots indicate $CFSE^{+}$ splenocytes proliferation when co-cultured with myeloid cells at indicated ratios; shaded indicates control CFSE dilution of splenocytes when cultured alone. Division index is the average number of cell divisions that a cell in the original population has undergone, which was calculated using FlowJo and normalized to splenocyte alone group in each experiment (**C**,**H**–**K**). Numbers in flow cytometric plots indicate the proportions of gated cells (**A**,**C**,**H**–**K**). Data are from two experiments (**A** and **C**–**E**; $N = 2$–4 per group in each experiment; mean and s.e.m.), representative of 3–5 mice per group (**B**,**F**,**G**), or representative of three experiments with cells pooled from 3 mice (**H**–**K**).



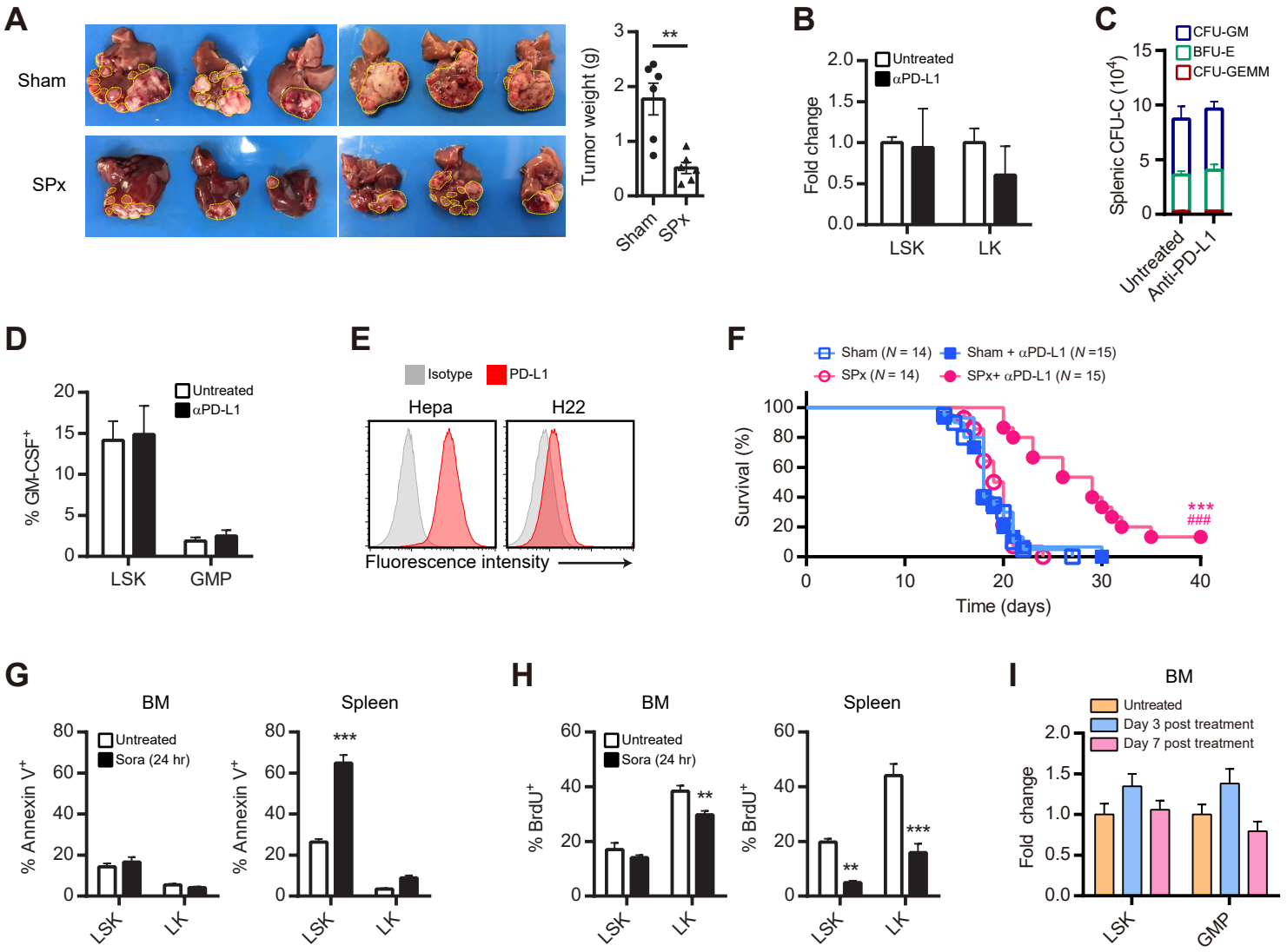
Supplemental Figure 4. Spleens of Hepa mice recruit and support HSPCs to produce myeloid suppressors. (**A**) Cartoon depicting the generation of control (N = 3 per group in each experiment) or Hepa-bearing (N = 4 per group in each experiment) parabiotic pairs. i.h., intrahepatic. (**B** and **C**) Quantification of EGFP$^+$ partner-derived splenic LSK and GMP cells (**B**), and number of EGFP$^+$ partner-derived CFU-C colonies (**C**) obtained from parabiotic mice pairs described in (**A**). Data are representative of two experiments, and presented as median and interquartile range (**B**), or mean and s.e.m. (**C**). $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant (**B**, two-way ANOVA on ranks, followed by Tukey's test; **C**, two-way ANOVA, followed by Tukey's test). (**D**) Scheme of the homing assay to assess the preference and efficiency of BM CFU-GM progenitors homing to the spleens of control or Hepa mice. i.h., intrahepatic. (**E**) Percentages of transferred BM-derived CFU-GM progenitors homing to the spleens of control (N = 7) or Hepa (N = 6) mice as described in (**D**). Data are from two experiments and presented as mean and s.e.m. $^{***}P < 0.001$ (Student's t-test). (**F**) The *Il6* mRNA expression in the spleens of control and Hepa mice (N = 7 per group). $^{***}P < 0.001$ (Student's t-test). (**G**) IL-6 release was measured in the supernatants of 48-hr cultures of indicated cell populations isolated from the spleens of control or Hepa mice (N = 3 per group; evaluated in triplicates), by ELISA. $^{*}P < 0.05$; $^{**}P < 0.01$ (two-way ANOVA followed by Bonferroni's test). (**H**) The endogenous GM-CSF expression of BM-derived LSK cells cultured in serum-free medium supplemented with SCF, TPO, and Flt3L (STF) for 4 days. Recombinant mouse IL-6 (50 ng/ml) was also added as indicated. Numbers in flow cytometric plots indicate the proportions of gated cells. Data are representative of two experiments.



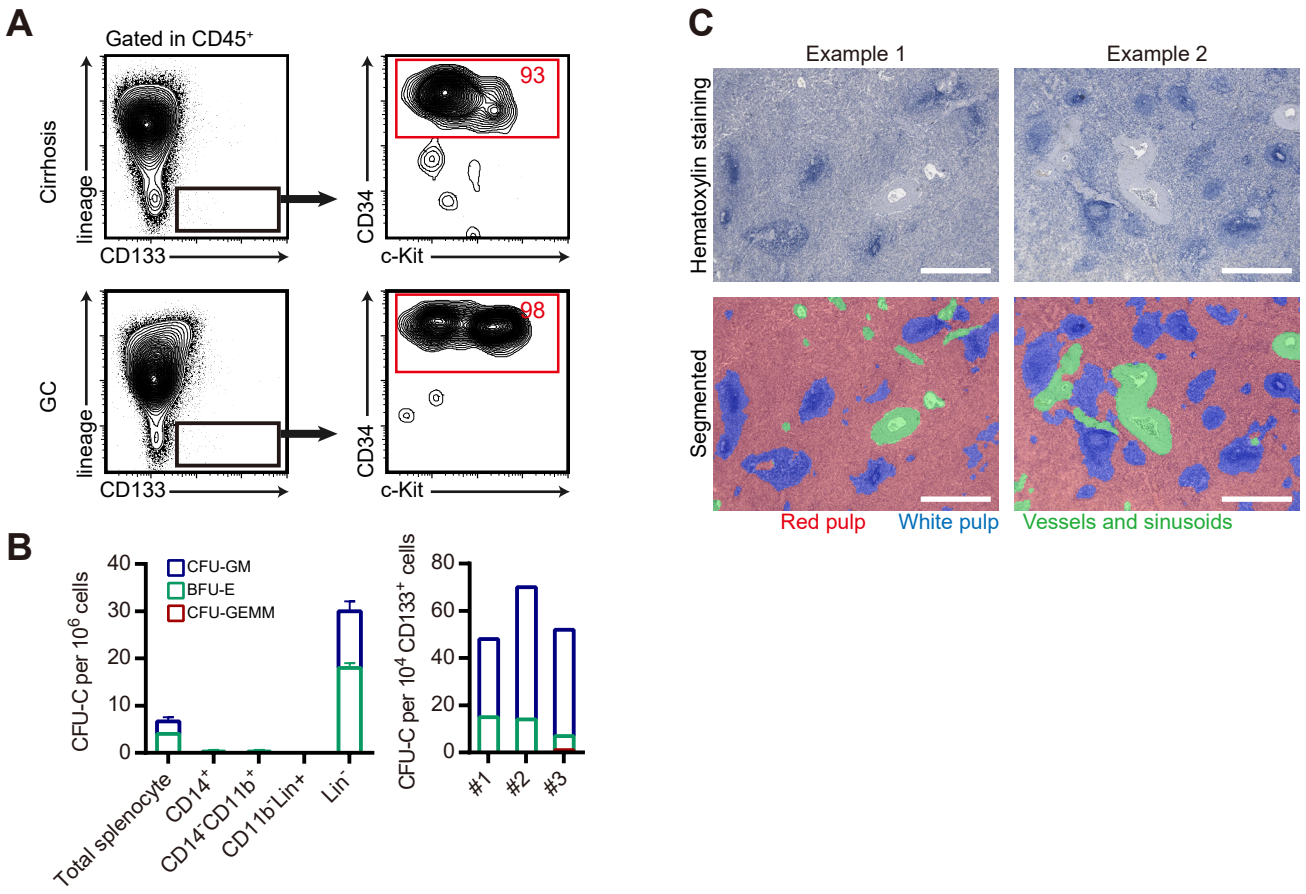
Supplemental Figure 5. Cancer induces a faster turnover and enhanced recruitment of splenic HSPCs. (**A**) As described in Supplemental Figure 4A, after four weeks of parabiosis, the proportions of EGFP$^+$ partner-derived LSK, LK, and Lin$^+$ splenocytes were analyzed by flow cytometry ($N = 6$ per group; mean and s.e.m.). $^{**}P < 0.01$ (two-way ANOVA followed by Bonferroni's test). (**B**) Tumor-free control ($N = 2$ for each time-point; mean and s.e.m.) or Hepa-bearing ($N = 3$ for each time-point; mean and s.e.m.) parabiotic pairs were surgically separated after four weeks of parabiosis. The proportions of EGFP$^+$ partner-derived splenic LSK and LK cells were analyzed at the indicated time points by flow cytometry. EGFP$^+$ chimerisms at 96 hr were compared to those at 0 hr within each group. $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant (two-way ANOVA followed by Dunnett's test). (**C** and **D**) The BrdU uptake of BM and splenic LSK cells from control or Hepa mice, after a 5 hr- or 24 hr-pulse. Data are representative of two experiments ($N = 5$–7 mice per group). $^{*}P < 0.05$; $^{***}P < 0.001$ (two-way ANOVA, corrected by Bonferroni's method). (**E**) The apoptotic rates of splenic HSPCs from control or Hepa mice. Data are representative of two experiments ($N = 5$ mice per group).


A
Control-1
Control-2
Hepa 2wk-1
Hepa 2wk-2
Hepa 4wk-1
Hepa 4wk-2
Ccl12
Ccl2
Mif
Cxcl1
Lefty1
Nampt
Spp1
Txlna
Osm
Inhba
Cxcl5
Il4
Tnfsf14
Ppbp
Il17f
Pf4
-1.5
1.5 (log 2 fold)
B
Control
Hepa
CXCL12+ cells per mm2 red pulp area (10^3)
***
Control
Hepa
C
Control
Hepa
Relative expression (fold)
**
***
**
Ccl2
Ccl7
Ccl8
Ccl12
Cxcl1
Cxcl2
Cxcl3
Pf4
Cxcl5
Ppbp
D
BM
Spleen
Isotype
Control
Hepa
Isotype
Control
Hepa
0.0
0.1
0.1
0.1
0.3
0.4
LSK
0.1
0.1
0.1
0.0
0.0
0.1
LK
CXCR2
CXCR4
E
Ccl2 relative expression (fold)
Control
Hepa
***
***
***
Total splenocyte
Splenic stroma
VE-cad+ stromal cell
VE-cad- stromal cell
Lymphocyte
PMN-like cell
Monocytic cell
F
Arg1
Relative expression level
*
CCR2+/+
CCR2-/-
G
CCR2+/+
CCR2-/-
% in CD45+
***
Monocytic
PMN-like

Supplemental Figure 6. Screening for factors mediating splenic recruitment of HSPCs in Hepa mice. (**A**) Heat map of the splenic cytokine and chemokine profiling by qPCR arrays. (**B**) Immunohistological detection of $CXCL12^+$ cells in the spleens of control and Hepa mice (N = 5–6 per group; mean and s.e.m.). Scale bar, 50 μm. ***$P < 0.001$ (Student's t-test). (**C**) The mRNA expression levels of *Ccl2*, *Ccl7*, *Ccl8*, *Ccl12*, *Cxcl1*, *Cxcl2*, *Cxcl3*, *Pf4*, *Cxcl5*, and *Ppbp* in the spleens of control (N = 6; mean and s.e.m.) and Hepa mice (N = 6; mean and s.e.m.). Values are relative to *Actb* mRNA expression, and normalized to the average levels in control spleens. **$P < 0.01$; ***$P < 0.001$ (two-way ANOVA followed by Bonferroni's test). (**D**) Flow cytometric analysis of the surface CXCR2 and CXCR4 expression on BM and splenic LSK and LK cells. Data are representative of 3–5 mice per group. Numbers in cytometric plots indicate the proportions of $CXCR2^+$ cells. (**E**) Fold changes in *Ccl2* mRNA expression levels in indicated cell populations isolated from the spleens of control or Hepa mice (N = 3 per group; mean and s.e.m.). ***$P < 0.001$ (two-way ANOVA followed by Bonferroni's test). (**F**) The *Arg1* mRNA expression, relative to *Actb*, in the tumor $CD11b^+Ly6G^+Ly6C^{low}$ PMN-like cells (N = 3–5 per group; mean and s.e.m.). *$P < 0.05$ (Student's t-test). (**G**) Frequencies of MDSC subsets in the tumors of $CCR2^{+/+}$ or $CCR2^{-/-}$ Hepa mice (N = 9 per group; mean and s.e.m.). ***$P < 0.001$ (Student's t-test).



Supplemental Figure 7. Abrogation of splenic EMH synergistically enhances anti–PD-L1 therapy. (**A**) Tumor sizes 4.5 weeks after 2×10^5 Hepa1-6 tumor cells were subcapsularly transplanted into the left lobe of livers. $^{**}P < 0.01$ (Mann-Whitney test). (**B**–**D**) The fold-changes of LSK and LK HSPCs (**B**), the CFU-C numbers (**C**), and the GM-CSF$^+$ proportions in the LSK and GMP subsets (**D**) in the spleens of Hepa mice treated with or without anti–PD-L1 ($N = 3$ per group; mean and s.e.m.). $P > 0.05$ (Student's t-test). (**E**) The PD-L1 expression on Hepa1-6 and H22 mouse hepatoma cell lines was determined by flow cytometry. Data are representative of two experiments. (**F**) Survival of H22 tumor-bearing mice subjected to a sham surgery (Sham) or splenectomy (SPx) with or without anti–PD-L1 (αPD-L1) treatment. Data are pooled from two experiments ($N = 14$–15 per group for each experiment as indicated). $^{***}P < 0.001$ compared to "SPx" group (log-rank test); $^{###}P < 0.001$ compared to "Sham + αPD-L1" group (log-rank test). (**G** and **H**) The survival (**G**) and proliferation (**H**; 5 hr-pulse) status of BM and splenic HSPCs in Hepa mice with or without low-dose sorafenib treatment ($N = 5$ mice per group; mean and s.e.m.). $^{***}P < 0.001$; $^{**}P < 0.01$ (two-way ANOVA corrected by Bonferroni's method). (**I**) Fold changes in the numbers of LSK and GMP cells in the BM of Hepa mice after low-dose sorafenib treatment. Data are representative of two experiments ($N = 3$ per group in each experiment; mean and s.e.m.). $P > 0.05$ (two-way ANOVA).



Supplemental Figure 8. Splenic EMH in human patients. (**A**) The phenotype of human nucleated $CD133^+$ splenocytes was examined by flow cytometry. $CD45^+lin^-CD133^+$ cells were gated, and their expression of CD34 and c-Kit was analyzed. Representative results of three cirrhosis patients and three gastric cancer (GC) patients are shown. Numbers in plots indicate the proportions of gated cells. (**B**) Numbers of CFU-C colonies obtained with indicated numbers of splenocytes. Data from one cirrhosis patient (#1) and two gastric cancer patients (#2 and #3) were pooled (left), or shown individually (right). BFU-E: Burst-forming unit-erythroid; CFU-GM: Colony-forming unit-granulocyte/macrophage; CFU-GEMM: Colony-forming unit-granulocyte/erythrocyte/macrophage/megakaryocyte. (**C**) Human spleen areas were automatically segmented and evaluated by a machine-learning strategy using InForm Software. Two examples of area segmentation are shown. Scale bar, 500 μm.

Supplemental Table 1. Clinical characteristics of the 169 patients contributing spleen samples

Patient characteristics	No. of patients	Age, years (median, range)	Sex (male, female)	TNM stage (I+II, III+IV)
Tumor type				
HCC	22	49, 32–65	19, 3	22, 0
Gastric cancer	86	59, 33–82	62, 24	16, 70
Kidney cancer	16	66.5, 35–73	10, 6	4, 12
Pancreatic cancer	11	57, 47–81	10, 1	3, 8
Non-cancer patients				
Splenic hemangioma	6	50, 32–67	1, 5	N/A
Cirrhosis	28	44, 33–63	23, 5	N/A

Abbreviations: HCC, hepatocellular carcinoma; N/A, not applicable.

Supplemental Table 2. Fluorochrome-conjugated antibodies used in flow cytometry

Antigen	Specificity	Fluorochrome	Clone	Supplier
BrdU		FITC	B44	BD Bioscience
CCR2	Mouse	PE	475301	R&D Systems
CD11b	Mouse	PE-Cy7	M1/70	BD Bioscience
CD16/CD32	Mouse	PE	2.4G2	BD Bioscience
CD3	Mouse	PE	17A2	Biolegend
CD8a	Mouse	eFluor 450	53-6.7	eBioscience
CD34	Mouse	Alexa Fluor 647	RAM34	BD Bioscience
CD45	Mouse	Brilliant Violet 570	30-F11	Biolegend
CD45.1	Mouse	FITC	A20	Biolegend
CD45.2	Mouse	PE-Cy7	104	Biolegend
CD117	Mouse	PE-Cy7	2B8	BD Bioscience
CD127	Mouse	PE-Cy5.5	A7R34	eBioscience
CD135	Mouse	PE-CF 594	A2F10.1	BD Bioscience
CD274	Mouse	PE-Cy7	10F.9G2	Biolegend
CXCR2	Mouse	PE	242216	R&D Systems
CXCR4	Mouse	Brilliant Violet 421	L276F12	Biolegend
GM-CSF	Mouse	Brilliant Violet 421	MP1-22E9	BD Bioscience
Gr-1	Mouse	APC	RB6-8C5	Biolegend
IFNγ	Mouse	Alexa Fluor 488	XMG1.2	eBioscience
Lineage Cocktail	Mouse	PerCP-Cy5.5	145-2C11,M1/70,RA3-6B2,TER-119,RB6-8C5	BD Bioscience
Ly-6C	Mouse	Brilliant Violet 421	HK1.4	Biolegend
Ly-6G	Mouse	PE-CF 594	1A8	BD Bioscience
Sca-1	Mouse	Alexa Fluor 700	D7	eBioscience
Rat IgG2b κ	Isotype ctrl	PE	eB149/10H5	eBioscience
Rat IgG2b κ	Isotype ctrl	PE-Cy7	RTK4530	Biolegend
CD34	Human	PE-Cy7	4H11	eBioscience
CD45	Human	Krome Orange	J.33	Beckman Coulter
CD117	Human	APC	104D2	eBioscience
CD133	Human	PE	CD133/2	Miltenyi
Lineage cocktail 4	Human	FITC	RPA-2.10, HIT3a, RPA-T4, M-T701, HIT8a, ICRF44, M5E2, SJ25-C1, 2H7, B159	BD Bioscience

Abbreviations: PE, phycoerythrin; FITC, fluorescein isothiocyanate; APC, allophycocyanin.

Supplemental Table 3. Gene-specific primers used for real-time PCR

Gene Name	Primer Sequence(5'–3')
Ccl2	Forward: CCAGCAAGATGATCCCAATGAGT
	Reverse: CATTCCTTCTTGGGGTCAGC
Ccl7	Forward: AGGATCTCTGCCACGCTTC
	Reverse: CGCAGACTTCCATGCCCTT
Ccl8	Forward: CAGATAAGGCTCCAGTCACC
	Reverse: TACACAGAGAGACATACCCTGC
Ccl12	Forward: CCTCCTGCTCATAGCTACCAC
	Reverse: AGCACAGATCTCCTTATCCAGT
Cxcl1	Forward: TTGCCTTGACCCTGAAGCTC
	Reverse: TCAGAAGCCAGCGTTCACC
Cxcl2	Forward: CCAGACAGAAGTCATAGCCACT
	Reverse: TCCTTTCCAGGTCAGTTAGCC
Cxcl3	Forward: CAGACAGAAGTCATAGCCACT
	Reverse: TTCCTCCTTTCCCGGCTCA
Pf4	Forward: GTGTGTGAAGACCATCTCCTC
	Reverse: GCAGCTGATACCTAACTCTCC
Cxcl5	Forward: GCCCTACGGTGGAAGTCATA
	Reverse: GTGCATTCCGCTTAGCTTTC
Ppbp	Forward: CTGCCCACTTCATAACCTCC
	Reverse: ACATCAGCACAGTGAACTCC
Cxcl12	Forward: CAGAGCCAACGTCAAGCATC
	Reverse: TTGTTCTTCAGCCGTGCAAC
Csf1	Forward: AGACTTCATGCCAGATTGCCT
	Reverse: GTTGTCTTTAAAGCGCATGGTC
Csf2	Forward: CAAAGAAGCCCTGAACCTCC
	Reverse: TGAAATTGCCCCGTAGACCC
Csf3	Forward: AGCCCAGATCACCCAGAATCC
	Reverse: GCCTCTCGTCCTGACCATAGT
IL6	Forward: AAGACAAAGCCAGAGTCCT
	Reverse: GTCTTGGTCCTTAGCCACT
Actb	Forward: CCAGGTCATCACTATTGGCAAC
	Reverse: TACGGATGTCAACGTCACAC

SUPPLEMENTAL METHODS

Animals. Wild-type C57BL/6 (B6) and BALB/c mice (6–8 weeks of age) were purchased from Guangdong Medical Laboratory Animal Center (Guangzhou, China). For the N-nitrosodiethylamine (DEN)-induced hepatoma model, male C57BL/6 mice (6–8 weeks of age) were purchased from Beijing Vital River Laboratory Animal Technology (Beijing, China). C57BL/6-Tg(CAG-EGFP)C14-Y01-FM131Osb (referred to as $EGFP^{+}$) and female C57BL/6JNju-*Apc*^{MinC}/Nju heterozygous (referred to as *Apc*$^{Min/+}$) mice were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China). B6.SJL-Ptprca Pepcb/BoyJ (referred to as BoyJ or $CD45.1^{+}$) mice, B6.129S4-Ccr2tm1Ifc/J (referred to as $CCR2^{-/-}$) homozygotes, C57BL/6-Tg(TRAMP)8247Ng/J heterozygotes (referred to as TRAMP), and C57BL/6-Tg(TcraTcrb)1100Mjb/J (referred to as OT-I) mice were purchased from the Jackson Laboratory. Mice were randomly divided into groups. Sample sizes were chosen on the basis of previous experience in the laboratory.

Supplemental tumor models. For DEN-induced hepatoma model, B6 mice received a single dose of DEN (1 mg/kg, i.p.), followed by repeated administration of CCl_4 (0.2 ml/kg, i.p.) beginning at 8 weeks of age for up to 14 consecutive weeks. Age-matched B6 male mice without DEN administration were used as controls. Mice were sacrificed at 10 months of age. For orthotopic lung cancer, 5×10^5 Lewis lung carcinoma (3LL) cells suspended in 100 μl PBS were transplanted into B6 mice by intratracheal instillation. For the melanoma model, 1×10^5 B16-F10 cells were injected subcutaneously into the left flank of B6 mice. The indicated numbers of tumor cells formed a tumor with 1.0–1.5 cm diameter within 4 weeks after implantation. H22 ascitic tumor was generated by transplanting 1×10^5 H22 tumor cells intraperitoneally into BALB/c mice. Cell lines were obtained from ATCC or China

Infrastructure of Cell Line Resources and tested free from mycoplasma contamination. Only the male mice were used to establish transplantable tumor models. TRAMP mice spontaneously developed prostate cancer by 24–30 weeks. Female *Apc*$^{Min/+}$ mice spontaneously developed intestinal adenomas by 15–17 weeks.

Competitive reconstitution assay. A total of 1×10^6 BM cells or 1×10^7 splenocytes from CD45.2$^+$ mice were mixed with 1×10^6 rescue BM cells from CD45.1$^+$ mice, and intravenously transferred into lethally irradiated (9.5 Gy) CD45.1$^+$CD45.2$^+$ recipient mice. Blood samples of recipients were collected from the tail vein every two weeks up to 16 weeks, and the percentages of donor-derived leukocytes were determined by flow cytometry.

Patients and specimens. Paraffin-embedded spleen samples from six patients with splenic hemangioma who underwent a splenectomy between August 1999 and April 2014, 28 patients with cirrhosis who underwent a splenectomy between February 2013 and July 2015, 22 patients with hepatocellular carcinoma who underwent a splenectomy between March 2005 and December 2015, 86 patients with gastric cancer who underwent a splenectomy between May 2000 and November 2011, 16 patients with kidney cancer who underwent a splenectomy between March 2000 and September 2014, and 11 patients with pancreatic cancer who underwent a splenectomy between February 2004 and December 2014 were used for immunohistochemical analysis. Details of patient information are provided in the **Supplemental Table 1**. Fresh spleen samples from three patients with cirrhosis who underwent a splenectomy between April 2014 and August 2016, and three patients with gastric cancer who underwent a splenectomy between April 2014 and October 2016 were used for splenocyte isolation. Isolated splenocytes were used for flow cytometry analysis and/or CFU-C assay.

Flow cytometry and cell isolation. For apoptosis analysis, freshly isolated cells were suspended with Annexin V-binding buffer. Cells were stained with Annexin V (Biovision) along with antibodies against surface markers and analyzed in this buffer. For intracytoplasmic GM-CSF detection, c-Kit$^+$ HSPCs were first isolated by MACS, and then stimulated in Serum-Free Medium (StemSpan SFEM; Stem Cell Technologies) supplemented with 50 ng/ml SCF and 0.5% Leukocyte Activation Cocktail (BD Pharmingen) at 37°C for 6 hr. For intracytoplasmic IFNγ detection, cells were stimulated in RPMI 1640 supplemented with 10% FBS (Gibco) and 0.5% Leukocyte Activation Cocktail at 37°C for 4.5 hr. After in vitro stimulation, cells were stained with antibodies against surface markers, and then fixed and permeabilized with Fixation/Permeabilization Solution Kit (BD Cytofix/Cytoperm), followed by staining with antibodies against GM-CSF or IFNγ.

For the isolation of HSPCs, lin$^{low/-}$ or c-Kit$^+$ cells were first sorted by MACS using the Lineage Cell Depletion Kit (Miltenyi) or CD117 microbeads (Miltenyi). Then, lin$^{low/-}$c-Kit^{high} HSPCs, lin$^{low/-}$c-Kit^{high}Sca-1$^+$ (LSK) cells, lin$^{ow/-}$c-Kit^{high}Sca-1$^-$ (LK) cells, or lin$^{low/-}$c-Kit^{high}Sca-1$^-$ FcγR^{high}CD34$^+$ GMPs were purified using a MoFlo XDP flow cytometer (Beckman Coulter). CD11b$^+$Ly6G$^-$Ly6C^{high} monocytic cells and CD11b$^+$Ly6G$^+$Ly6C^{low} PMN-like cells were purified by FACS. To isolate splenic stromal cells, spleen cells were isolated using an enzyme-based digestion method. Lin$^-$ spleen cells were first sorted by MACS using the Lineage Cell Depletion Kit (Miltenyi), and then incubated with mouse CD45 and CD117 microbeads (Miltenyi Biotec). CD45$^-$c-Kit$^-$lin$^-$ splenic stromal cells were purified by MACS. To further isolate splenic endothelial cells, total splenic stromal cells were incubated with anti–CD144-biotin (Biolegend), and then with anti-biotin microbeads (Miltenyi) for MACS. The purity of the sorted cell populations was evaluated by flow cytometry and exceeded 90%.

Immunohistochemistry. Paraffin-embedded samples were cut into 3 to 4-μm sections, which were processed for immunohistochemistry. Paraffin-embedded mouse tissue sections were de-paraffinized and rehydrated. The sections were then incubated in 0.3% hydrogen peroxide solution, prior to heat-induced epitope retrieval by citrate buffer (pH 6.0). For mouse c-Kit staining, sections were incubated with 5% BSA (Sigma-Aldrich) for blocking and then incubated with mouse SCF R/c-kit antibody (polyclonal goat IgG, 1:2000; R&D Systems) at 4°C overnight. The sections were stained with a Polink-2 plus HRP Goat with DAB Kit (GBI Labs). For mouse CXCL12 staining, the sections were incubated with 2.5% normal horse serum for blocking (Vector Laboratories) and then incubated with anti-SDF1 antibody (polyclonal rabbit IgG, 1:2000; Abcam) at 4°C overnight. The sections were stained with an ImmPRESS HRP Anti-Rabbit IgG (Peroxidase) Polymer Detection Kit (Vector Laboratories) and DAB (Dako). For human spleen sections, a CD133 antibody (PROM1 polyclonal antibody, 1:100; Abnova) or a CD11b antibody (EPR1344, 1:1000; Abcam) was applied to paraffin-embedded spleen sections after Tris-HCl (pH 9.2)- or citrate buffer-mediated heat-induced epitope retrieval, respectively. The sections were stained with an Envision System (Dako). All sections were counterstained with Harris hematoxylin solution (ZSGB-Bio) and mounted with balsam neutral (ZSGB-Bio).

Induction of EMH by bleeding. Three-month-old mice were bled via the tail vein four times with intervals of three days, removing approximately 300 μl of blood each time. Mice were sacrificed one day after the last bleeding for analysis.

Cell culture. HSPCs were cultured in Serum-Free Expansion Medium (StemSpan SFEM, Stem Cell Technologies) supplemented with recombinant mouse GM-CSF (50 ng/ml), SCF (50 ng/ml), or IL-6 (50 ng/ml) as indicated. For LSK cells and stromal cells co-culture, 5×10^4/ml naïve BM LSK cells were co-cultured with 5×10^4/ml $lin^-CD45^-c\text{-}Kit^-$ stromal cells of indicated origins in IMDM + 10% FBS complete medium, supplemented with SCF (50 ng/ml), TPO (10 ng/ml), and Flt3L (100 ng/ml), for 4 days. For transwell assays, BM-derived LSK cells were incubated for 4 days in the upper chamber of a transwell with splenic stromal cells from Hepa mice in the lower chamber. In order to test ability of the cultured HSPCs to produce MDSCs, lin^- cells from the upper chamber were collected after the 4-day culture, sorted by MACS, and continued to be cultured for another 3 days in StemSpan SFEM supplemented with SCF (50 ng/ml). Then, the $Gr\text{-}1^+$ myeloid descendant cells were isolated and tested for their suppressive function on anti-CD3/28 stimulated T cell proliferation. In some experiments, anti–mouse GM-CSF (3 μg/ml unless indicated otherwise; eBioscience), anti–mouse IL-6 (1 μg/ml; eBioscience), or equal amounts of corresponding isotype control Abs were added to the cultures. Cells were cultured at 37°C in a 5% CO_2 humidified atmosphere.

Immunoblotting. 1×10^4 LSK cells or 2×10^5 myeloid cells were lysed with 10μl lysis buffer. Equal amounts of cellular proteins were separated by 10% SDS-PAGE, immunoblotted with the indicated Abs, and visualized using the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). Primary antibodies used for immunoblotting were as follows: β-actin (AC-15; Boster Biological Technology), t-p65 (D14E12; Cell Signaling Technology), p-p65 (93H1; Cell Signaling Technology), t-p38 (#9212; Cell Signaling Technology), p-p38 (D3F9; Cell Signaling Technology), t-STAT3 (124H6; Cell Signaling Technology), p-STAT3 (D3A7; Cell Signaling Technology), Arg1 (D4E3M; Cell Signaling Technology).

BrdU incorporation assay. To assess the cell cycle status of HSPCs, mice were intraperitoneally injected with a single dose of BrdU (1 mg BrdU per 6 g body weight). After a 5-hr or 24-hr pulse, c-Kit$^+$ HSPCs were isolated by MACS from the BM or spleen, and stained for cell surface markers. Next, the cells were fixed, permeablized and stained for BrdU using the FITC BrdU Flow Kit (BD Pharmingen) according to the manufacturer's instructions. DAPI (Sigma-Aldrich) was added in FACS buffer to a concentration of 3 μg/ml to detect the DNA content.

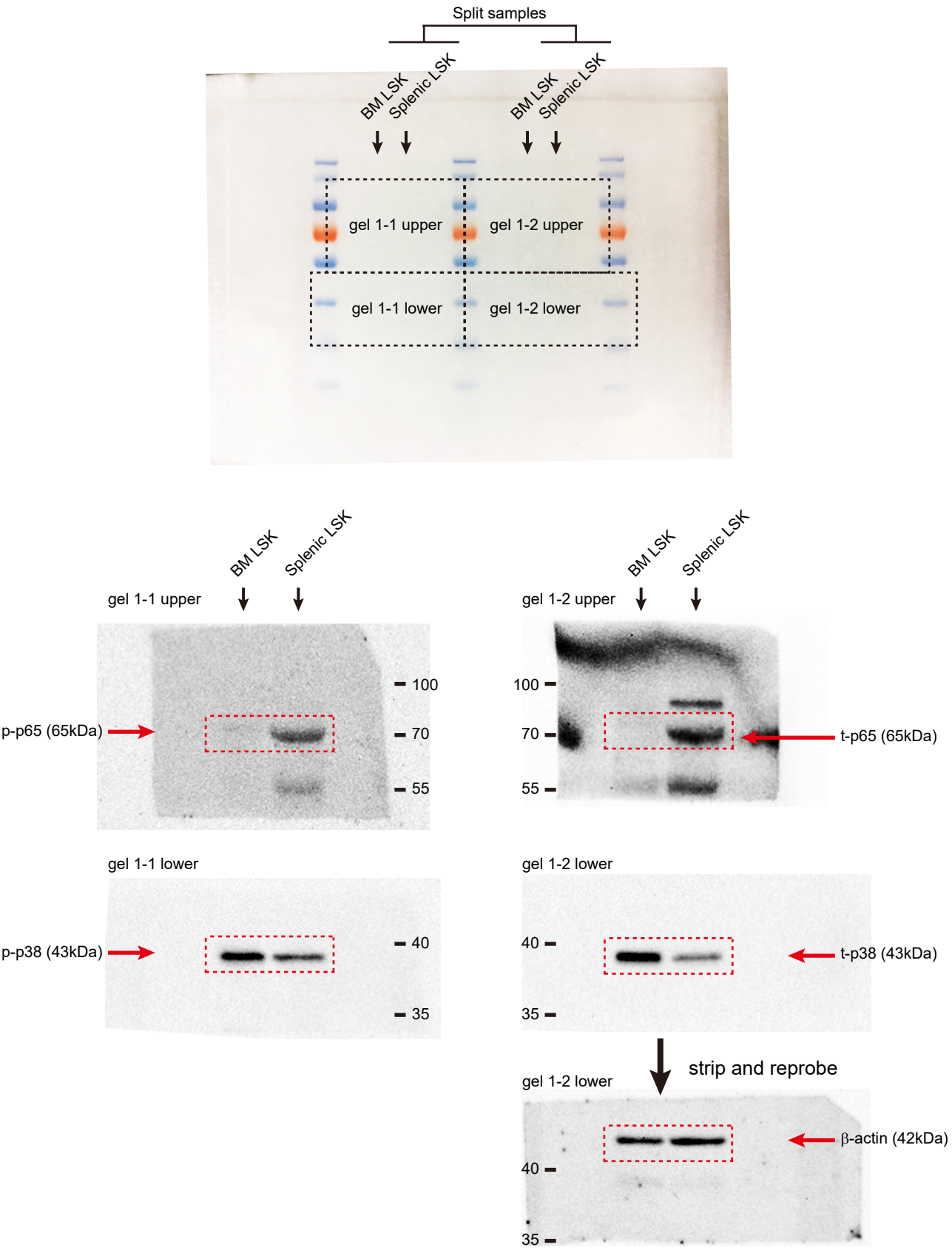
Splenectomy and parabiosis. Under anesthesia, the abdominal cavity of the mouse was opened, and the splenic vessels were cauterized. Then, the spleen was carefully removed. For sham surgery, the abdomen was opened but the spleen was not removed. For parabiosis experiments, sex- and weight-matched EGFP$^+$ and B6 mice (or CD45.1$^+$ BoyJ and CD45.2$^+$ B6 mice) were used. Paired mice were anesthetized and joined at the flanks. The percentage chimerisms for cell populations of interest were evaluated by flow cytometry or the colony forming cell assay.

HSPC intrasplenic transfer. 1×10^5 CD45.1$^-$ LSK cell from indicated origins were suspended in 30 μl HBSS, and subcapsularly injected into the spleens of CD45.1$^+$ recipient mice. After 5 days, splenocytes of the recipient mice were harvested and analyzed by flow cytometry. CD45.1$^-$Gr-1$^+$ donor-derived myeloid cells were sorted by MACS and tested for their suppressive activities.

Real-time PCR and quantitative PCR array. A total of 50–100 mg mouse spleen, liver, or tumor tissues were grinded with a mortar and pestle in liquid nitrogen, and homogenized in TRIzol Reagent (Ambion). Total RNA was extracted according to the manufacturer's instructions and quantified with a Nanodrop Instrument (Thermo Scientific). Aliquots containing 2 μg total RNA were used to generate complimentary DNA (cDNA) using the All-in-One RT MasterMix (Applied Biological Material). The target genes were evaluated by qPCR analysis using SYBR Green qPCR Master Mix (TOYOBO) or TaqMan Gene Expression Assays (Thermo Fisher Scientific). All reactions were run by a LightCycler 480 Instrument (Roche) in triplicate. TaqMan assays were used for evaluation of *Arg1* (Cat. No. Mm00475988), *Nos2* (Cat. No. Mm00440502_m1), and *Actb* (Mm01205647_g1) expression in PMN-like cells. Details of the gene-specific primers used for real-time PCR are provided in the **Supplemental Table 3**. For quantitative PCR array profiling, the cDNA of common cytokines and chemokines were quantified using the RT² Profiler PCR array-mouse common cytokines (Cat. No. PAMM-021Z, SABioscience) and RT² Profiler PCR array-mouse chemokines & receptors (Cat. No. PAMM-022Z, SABioscience) assay panels. Data were analyzed using the ΔΔCt method. Array data were further processed, clustered, and visualized with Cluster 3.0 and Java TreeView. The array data are available from the Gene Expression Omnibus (GEO) database (GEO: GSE84248).

Enzyme-linked immunosorbent assay. GM-CSF (Cat. No. 88-7334; eBioscience), IL-6 (Cat. No. 88-7604; eBioscience), and CCL2 (Cat. No. 88-7391; eBioscience) were measured according to the manufacturer's instructions. For measurement of cytokine or chemokine release, a total of 1×10^6 splenocytes, splenic stromal cells, PMN-like cells, monocytic cells, or Gr-1$^-$CD45$^+$ splenic lymphocytes were cultured respectively in flat-bottom 96-well plates with 200 μl RPMI 1640 supplemented with 10% FBS for 48 hr. The levels of cytokines and chemokines in the supernatants of cultures were measured in triplicates.

Full unedited gel for Figure 2B



Full unedited gel for Supplemental Figure 3G