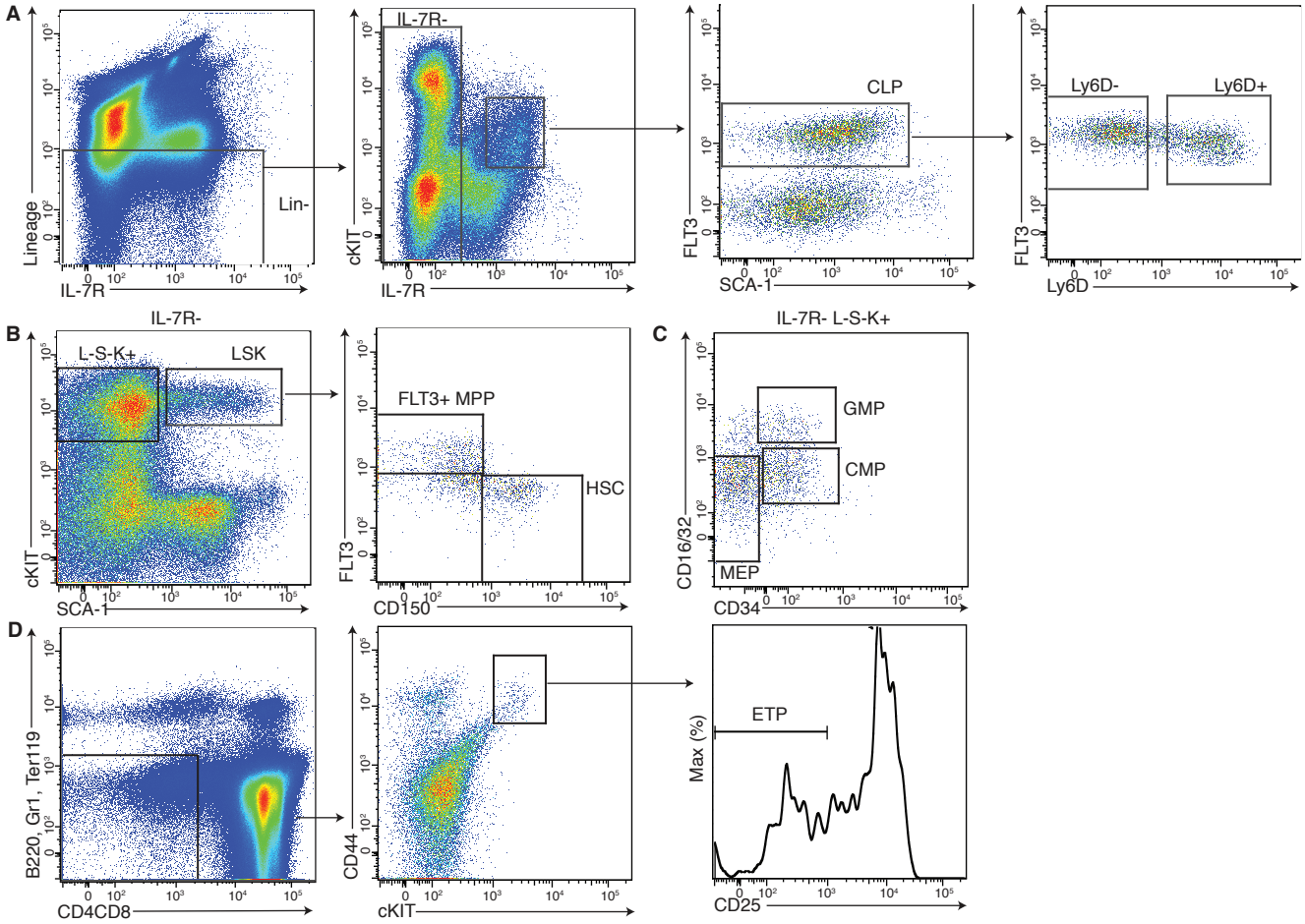

A
Lineage
IL-7R
Lin-
IL-7R-
cKIT
IL-7R
CLP
FLT3
SCA-1
Ly6D-
Ly6D+
FLT3
Ly6D
B
IL-7R-
L-S-K+
LSK
cKIT
SCA-1
FLT3+ MPP
HSC
FLT3
CD150
C
IL-7R- L-S-K+
GMP
CMP
MEP
CD16/32
CD34
D
B220, Gr1, Ter119
CD4CD8
CD44
cKIT
ETP
Max (%)
CD25

Figure S1

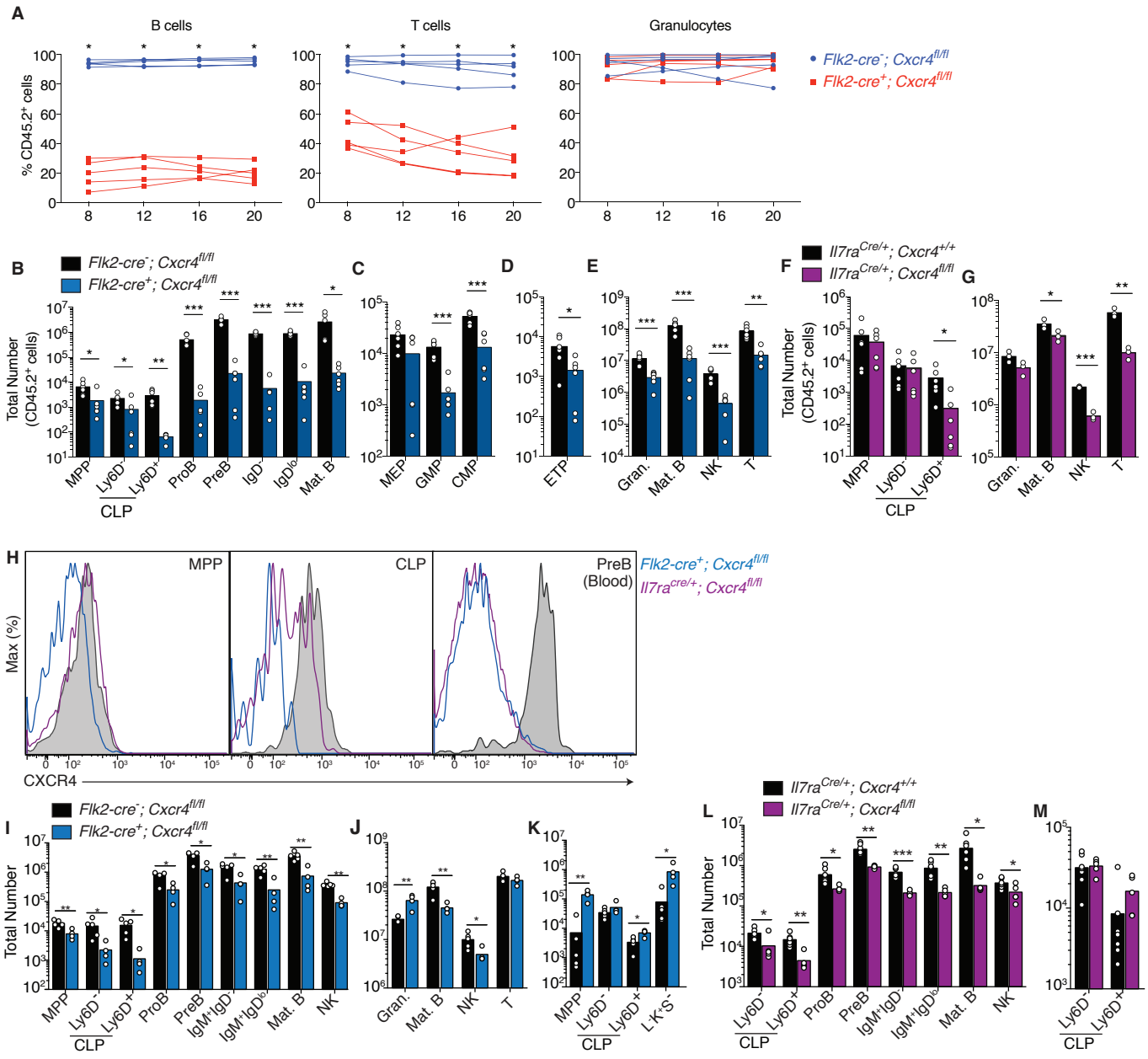
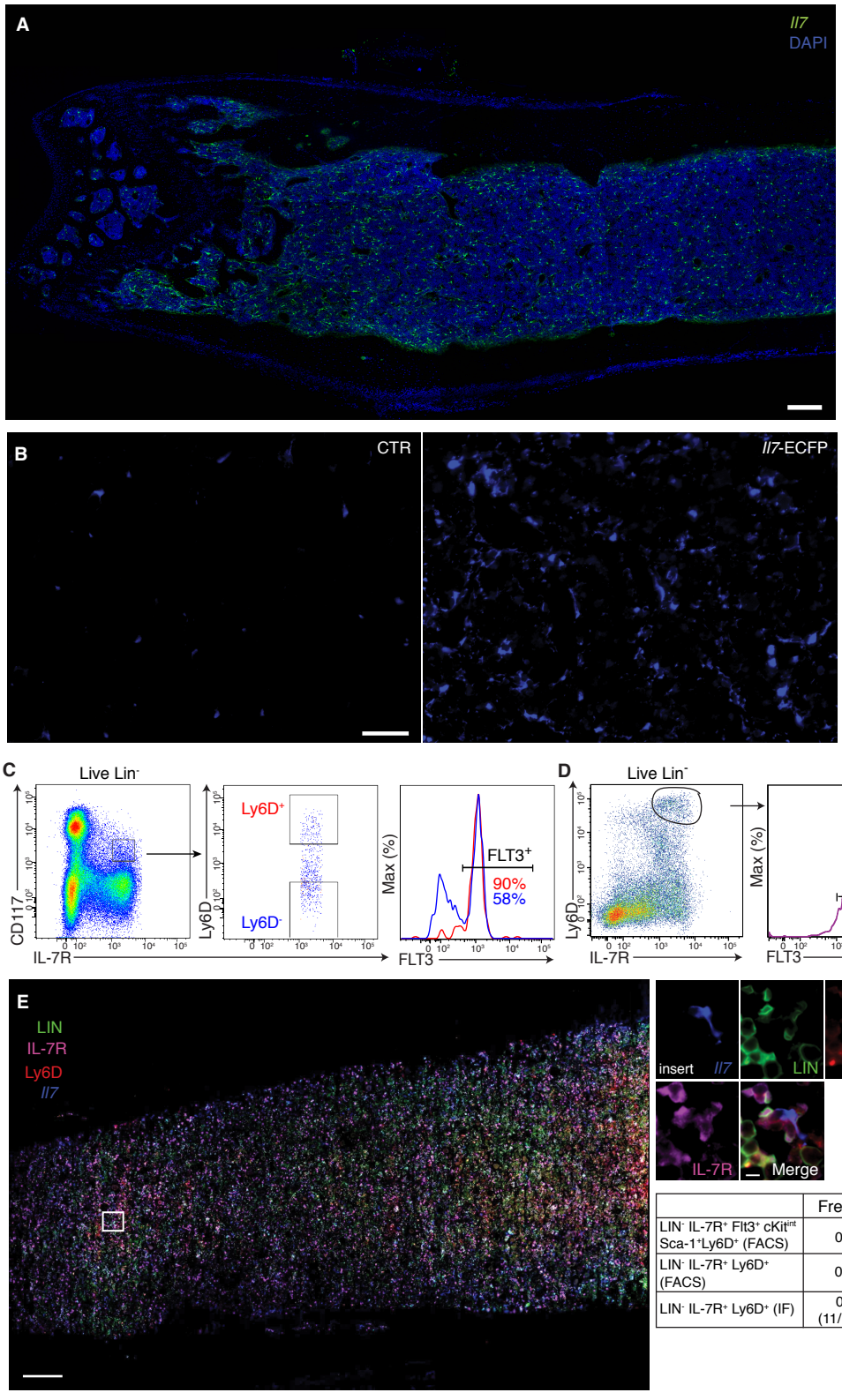

A
B cells
T cells
Granulocytes
% CD45.2+ cells
Flk2-cre-; Cxcr4fl/fl
Flk2-cre+; Cxcr4fl/fl
B
Total Number (CD45.2+ cells)
MPP
Ly6D-
Ly6D+
CLP
ProB
PreB
IgD-
IgDlo
Mat. B
C
MEP
GMP
CMP
D
ETP
E
Gran.
Mat. B
NK
T
F
Il7raCre/+; Cxcr4+/+
Il7raCre/+; Cxcr4fl/fl
G
H
MPP
CLP
PreB (Blood)
Max (%)
CXCR4
I
IgM+IgD-
IgM+IgDlo
J
K
L-K+S-
L
M

Figure S2



	Freq. (%)
LIN- IL-7R+ Flt3+ cKit^{int} Sca-1+Ly6D+ (FACS)	0.03
LIN- IL-7R+ Ly6D+ (FACS)	0.08
LIN- IL-7R+ Ly6D+ (IF)	0.07 (11/16064)

Figure S3

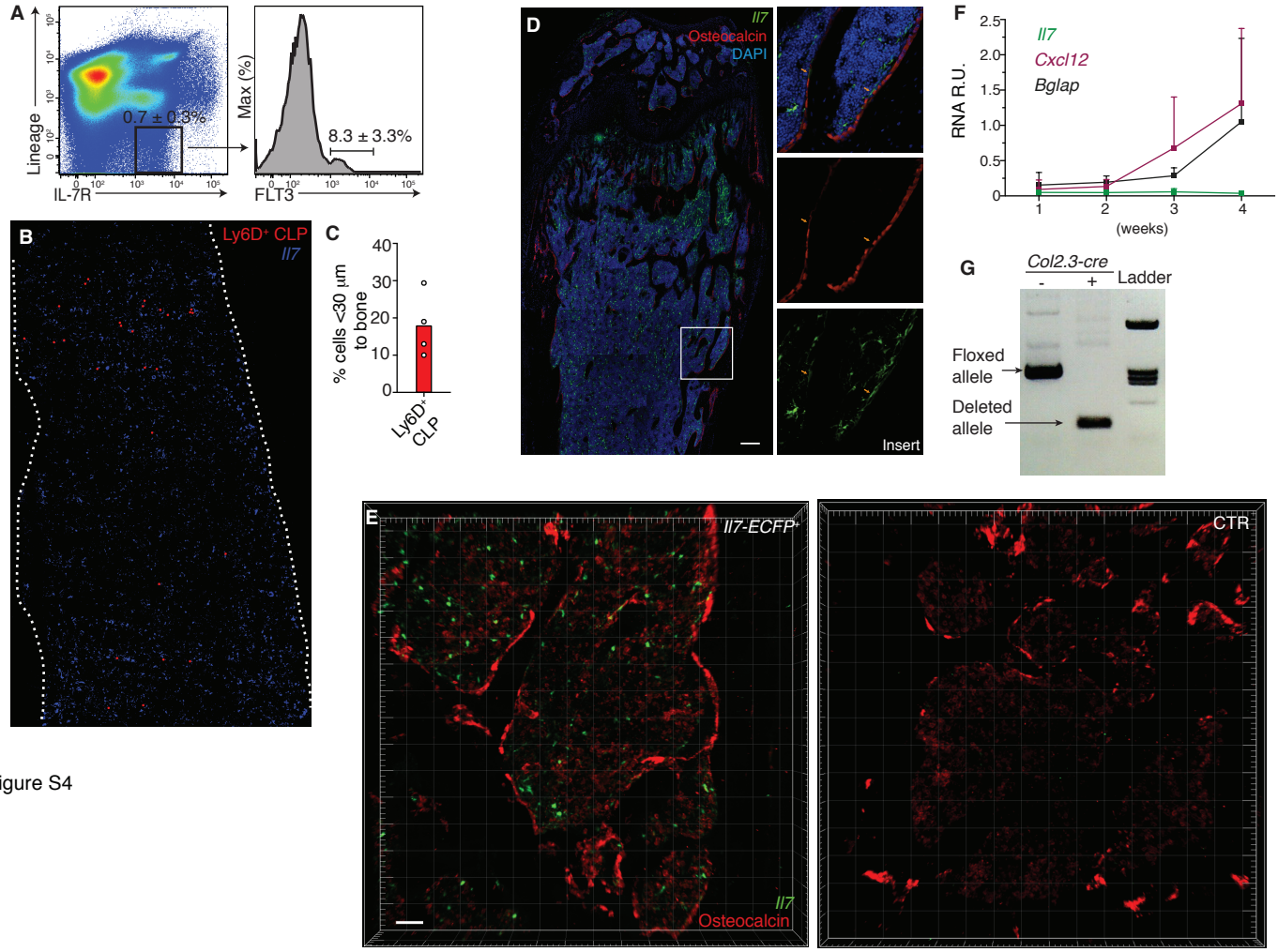

A
Lineage
IL-7R
0.7 ± 0.3%
Max (%)
FLT3
8.3 ± 3.3%
B
Ly6D+ CLP
Il7
C
% cells <30 µm to bone
Ly6D+ CLP
D
Il7
Osteocalcin
DAPI
Insert
F
RNA R.U.
Il7
Cxcl12
Bglap
(weeks)
G
Col2.3-cre
-
+
Ladder
Floxed allele
Deleted allele
E
Il7-ECFP
Il7
Osteocalcin
CTR

Figure S4

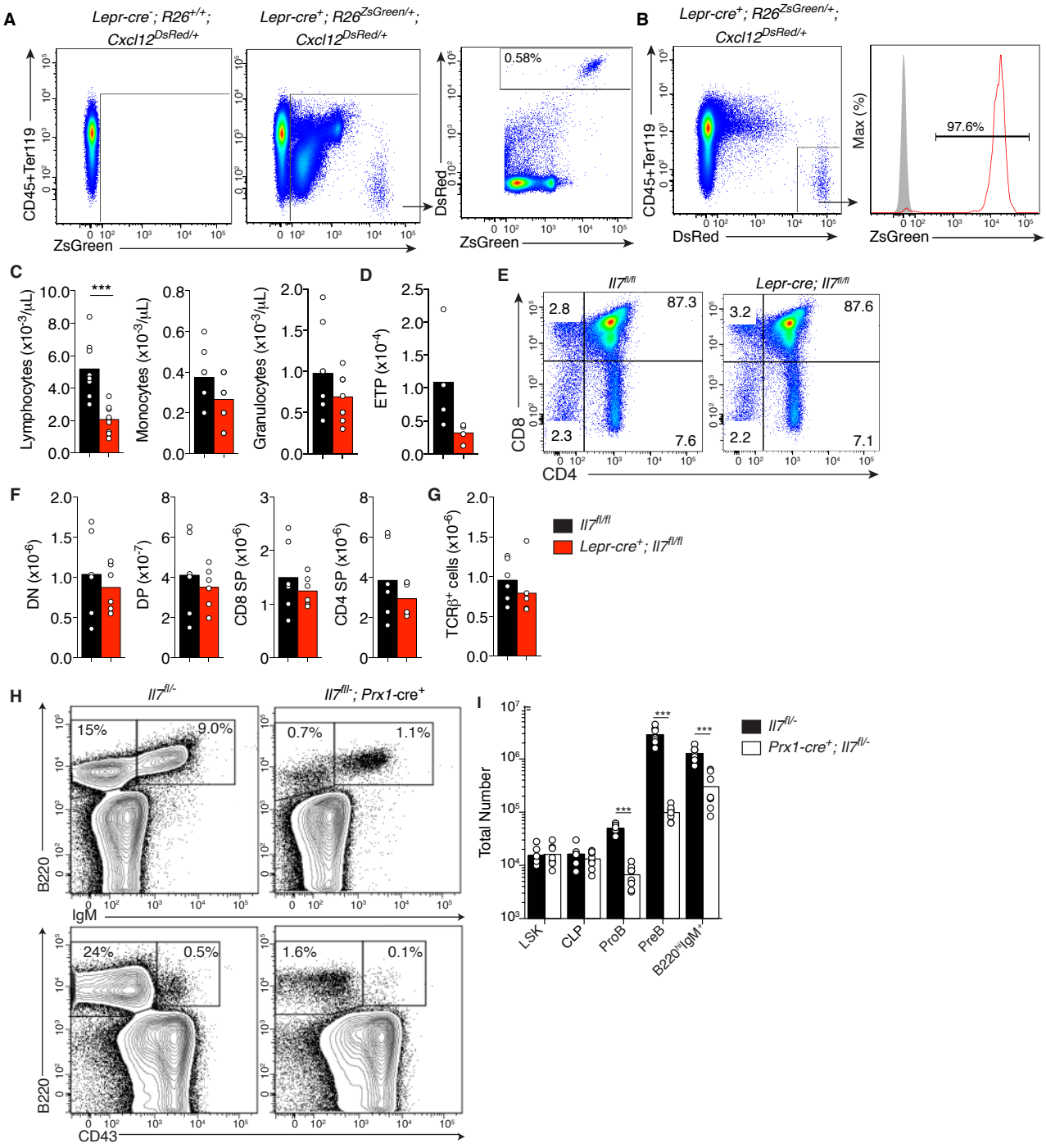

A
Lepr-cre-; R26+/+; Cxcl12DsRed/+
Lepr-cre+; R26ZsGreen/+; Cxcl12DsRed/+
CD45+Ter119
ZsGreen
DsRed
0.58%
B
Lepr-cre+; R26ZsGreen/+; Cxcl12DsRed/+
CD45+Ter119
DsRed
Max (%)
97.6%
ZsGreen
C
Lymphocytes (x10-3/μL)
Monocytes (x10-3/μL)
Granulocytes (x10-3/μL)
***
D
ETP (x10-4)
E
Il7fl/fl
Lepr-cre; Il7fl/fl
CD8
CD4
2.8
87.3
2.3
7.6
3.2
87.6
2.2
7.1
F
DN (x10-6)
DP (x10-7)
CD8 SP (x10-6)
CD4 SP (x10-6)
G
TCRβ+ cells (x10-6)
Il7fl/fl
Lepr-cre+; Il7fl/fl
H
Il7fl/-
Il7fl/-; Prx1-cre+
B220
IgM
15%
9.0%
0.7%
1.1%
CD43
24%
0.5%
1.6%
0.1%
I
Total Number
Il7fl/-
Prx1-cre+; Il7fl/-
LSK
CLP
ProB
PreB
B220hiIgM+

Figure S5

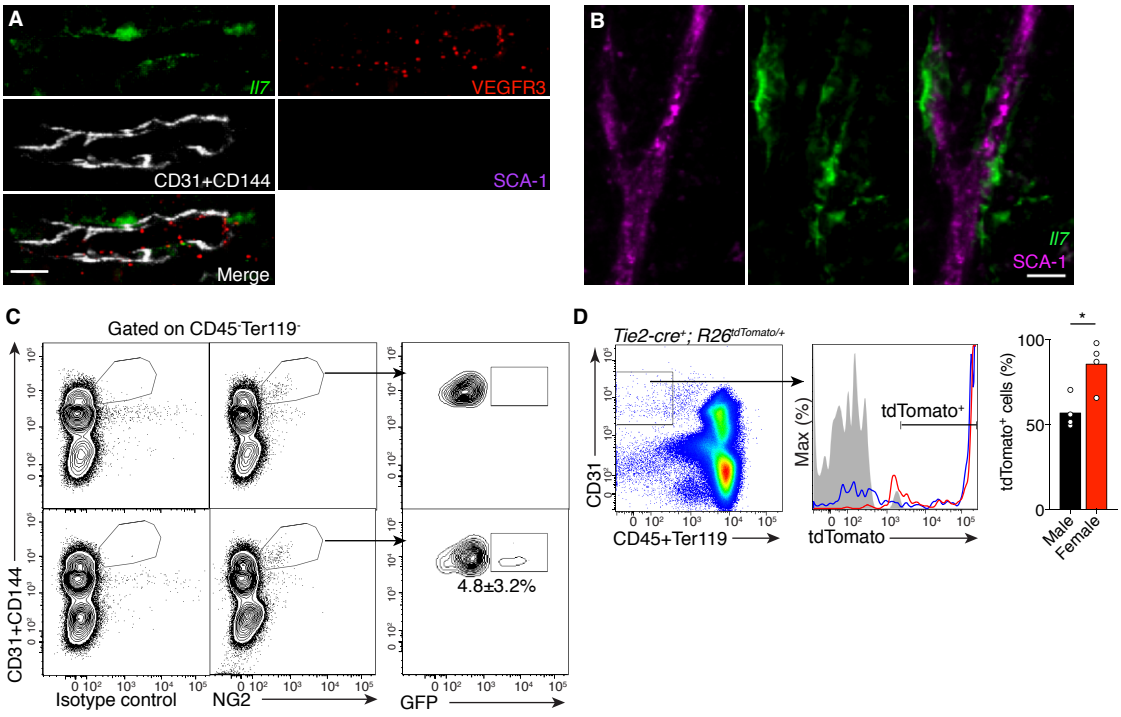

A
Il7
VEGFR3
CD31+CD144
SCA-1
Merge
B
Il7
SCA-1
C
Gated on CD45-Ter119-
CD31+CD144
Isotype control
NG2
GFP
4.8±3.2%
D
Tie2-cre+; R26tdTomato/+
CD31
CD45+Ter119
Max (%)
tdTomato+
tdTomato
tdTomato+ cells (%)
100
50
0
Male
Female
*

Figure S6

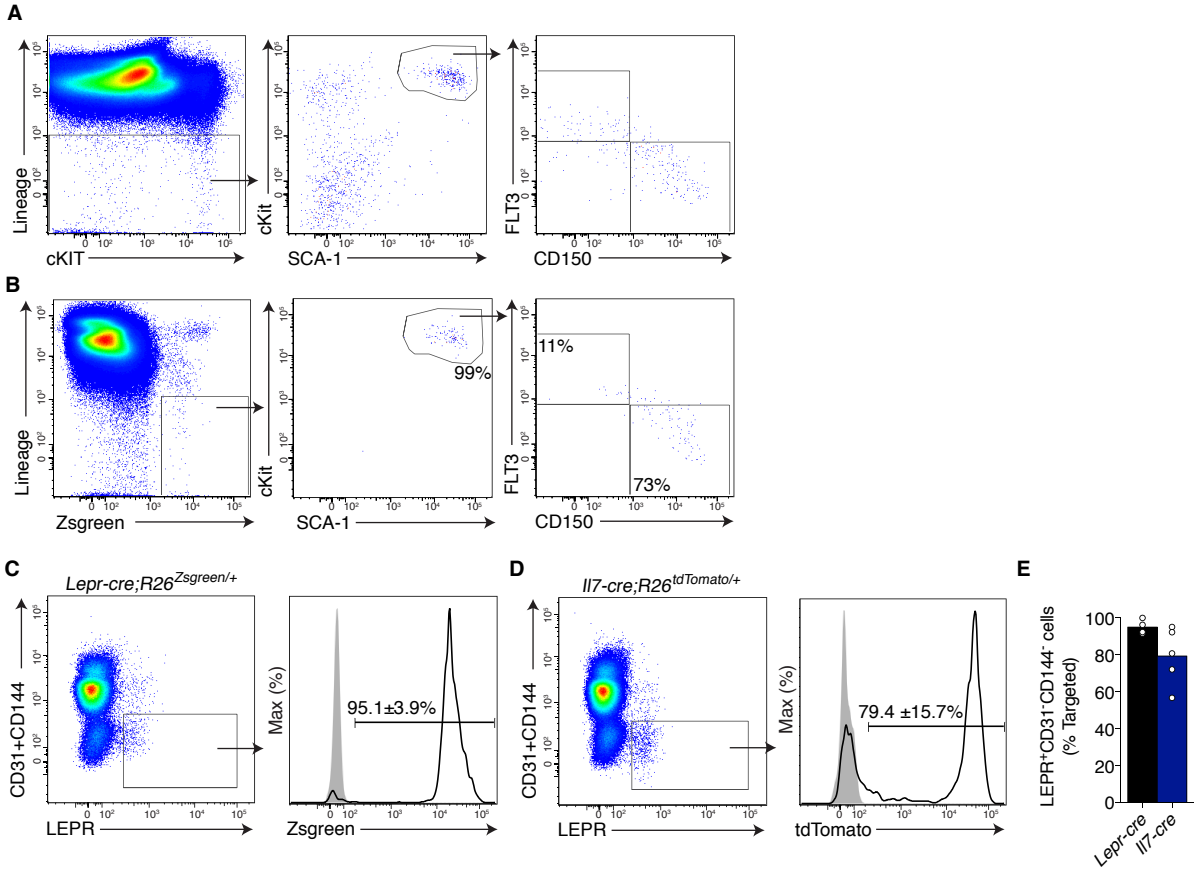

A
Lineage
cKIT
cKit
SCA-1
FLT3
CD150
B
Lineage
Zsgreen
cKit
SCA-1
99%
FLT3
CD150
11%
73%
C
Lepr-cre;R26Zsgreen/+
CD31+CD144
LEPR
Max (%)
Zsgreen
95.1±3.9%
D
Il7-cre;R26tdTomato/+
CD31+CD144
LEPR
Max (%)
tdTomato
79.4 ±15.7%
E
LEPR+CD31-CD144- cells
(% Targeted)
100
80
60
40
20
0
Lepr-cre
Il7-cre

Figure S7

Supplementary figure legends.

Figure S1, related to Figure 1: Gating strategy for analyzing HSCs and hematopoietic progenitor cell subsets. A, Common lymphoid progenitor subsets. **B**, Long-term HSCs and MPPs. Cells shown were gated on the indicated live ($DAPI^-$) LSK gate. **C**, MEP, CMP and GMP. Cells shown were gated on live ($DAPI^-$) $IL7R^- L^-S^-K^+$ gate. **D**, ETPs. Data are representative of more than 10 independent experiments.

Figure S2, related to Figure 1: CXCR4 deficiency at MPP and CLP stages reduces lymphoid cell differentiation. A, Frequency of $CD45.2^+$ hematopoietic cells in peripheral blood of mice reconstituted with 90% $CD45.2^+$ *Flk2-cre*$^-$*;Cxcr4*$^{fl/fl}$ (blue) or *Flk2-cre*$^+$*;Cxcr4*$^{fl/fl}$ (red) cells mixed with 10% $CD45.1^+$ WT cells 8-20 weeks after reconstitution. Lines connect individual mice, n=5 in each group. **B-E**, Number of $CD45.2^+$ hematopoietic cell subsets isolated from 90:10 *Flk2-cre*$^+$*;Cxcr4*$^{fl/fl}$ (blue) and *Flk2-cre*$^-$*;Cxcr4*$^{fl/fl}$ (black) mixed BM chimeras depicted in **Fig. 1A-D**. **B** and **C**, BM. **D**, thymus. **E**, combined lymphoid and non-lymphoid organs (BM, blood, spleen, LNs, peritoneal cavity, Peyer's patch, liver, and lung). **F** and **G**, Number of $CD45.2^+$ hematopoietic cell subsets isolated from 90:10 *Il7ra*$^{Cre/+}$*;Cxcr4*$^{fl/fl}$ mice (purple) and *Il7ra*$^{Cre/+}$*;Cxcr4*$^{+/+}$ mice (black) mixed BM chimeras depicted in **Figure 1E-G**. **F**, BM. **G**, combined lymphoid and non-lymphoid organs (BM, blood, spleen, LNs, peritoneal cavity, Peyer's patch, liver, and lung). **H**, Histogram of CXCR4 expression in gated MPP (left), CLP (middle), and preB (right) cells isolated from control (filled), *Flk2-cre*$^+$*;Cxcr4*$^{fl/fl}$ (blue), and *Il7ra*$^{cre/+}$*;Cxcr4*$^{fl/fl}$ (pink) mice. Data are representative of more than 3 independent experiments. **I-K**, Analysis of *Flk2-cre*$^+$*;Cxcr4*$^{fl/fl}$ (blue) and *Flk2-cre*$^-$*;Cxcr4*$^{fl/fl}$ (black) mice. **I**, Number of hematopoietic progenitor cell subsets, developing B cell subsets, and NK cells in the femur and tibia. **J**, Total number of granulocytes (gran.), mature B cells, NK and T cells in combined primary and secondary lymphoid organs, peritoneal cavity, and in liver. **K**, Number of hematopoietic cell subsets in the spleen. **L** and **M**, Analysis of *Il7ra*$^{Cre/+}$*;Cxcr4*$^{fl/fl}$ mice (purple) and *Il7ra*$^{Cre/+}$*;Cxcr4*$^{+/+}$ mice (black). **L**, Number of hematopoietic progenitor cell subsets, developing B cell subsets, and NK cells in the femur and tibia. **M**, Number of CLP subsets in the spleen. Bars indicate mean; circles depict individual mice. Data in all panels are representative of 3

independent experiments. * P<0.05, **P<0.01, ***P<0.001 (unpaired, two-tailed Student's t-test).

Figure S3, related to figure 2: Strategy for in situ detection of Ly6D$^+$ CLP cells. **A**, Femur whole mount confocal microscopy of *Il7*-ECFP cells (green) counterstained with DAPI (blue) The compiled image was assembled from individually scanned fields of view that were subsequently tiled manually. Scale bar is 200 μm. **B**, Distribution of IL-7$^+$ cells in femur 7-μm sections stained with anti-GFP antibodies. Left, control (CTR); right, *Il7*-ECFP transgenic mouse. **C**, FLT3 expression in Lineage$^-$,(Lin$^-$) cKit^{lo}, IL7Ra$^+$, Ly6D$^-$ and Ly6D$^+$ BM cells. Left panel shows Live Lin$^-$ gated BM cells. Middle panel shows Ly6D expression in live Lin$^-$ cKit^{lo} IL7Ra$^+$ cells. Right panel shows FLT3 expression Ly6D$^-$ and Ly6D$^+$ CLPs. **D**, Frequency of FLT3 expressing cells in gated live Lin$^-$ IL7Ra$^+$ and Ly6D^{hi} BM cells. Left panel shows Ly6D and IL-7Ra expression in total live Lin$^-$ BM cells; right panel shows FLT3 expression in gated population. **E**, 7-μm femur section of *Il7*-ECFP transgenic mice stained with anti-GFP (blue), lineage antibody cocktail (green), anti-IL-7Ra (purple), and with anti-Ly6D antibodies. Scale bar is 200 μm. White box in large panel indicates insert; individual fluorescence channels of magnified inserts are shown on the right. Data are representative of more than 10 independent experiments. Table, cell frequencies determined by immunofluorescence (IF) and by flow cytometry (FACS).

Figure S4, related to Figure 3: CLP gating strategy used by Ding and Morrison (2013), and CLP distribution in situ. **A**, Left panel, frequency of total IL-7Ra$^+$ Lin$^-$ cells in BM. Right panel, FLT3 expression in gated IL-7Ra$^+$ Lin$^-$ cells. Numbers indicate average ± SD (n=8 mice pooled from 3 independent experiments). **B**, Distribution of Ly6D$^+$ IL-7Ra$^+$ Lin$^-$ CLPs and IL-7$^+$ cells in a 7 μm-thick section of *Il7*-ECFP transgenic femur. Dotted line indicates bone surface. **C**, Frequency of Ly6D$^+$ IL-7Ra$^+$ Lin$^-$ CLPs positioned at < 30 μm from bone surface. Bars indicate average, circles depict individual mice. Data was pooled from 3 independent experiments. **D** and **E**, Distribution of IL-7$^+$ cells and osteocalcin$^+$ cells in femur whole bone mounts or 25 μm-thick sections. Scale bar is 200 μm in **D** and 50 μm in **E**. **F**, *Il7*, *Cxcl12*, and *Bglap*

expression in neonatal-derived calvaria osteoblasts cultured over a 4 week period (x-axis). Expression is relative to *Hprt* (y-axis). Lines indicate average ± SD of 3-independent experiments. **G**, *Il7* deletion in DNA isolated from osteoblasts differentiated in vitro from BM of *Col2.3*-Cre*;Il7*$^{fl/-}$ mice and control littermate.

Figure S5, related to Figure 4: IL-7 expressed by Lepr^{+} MSPCs is critical for B-lymphopoiesis. A and **B**, *Lepr*-cre fate mapping of CXCL12^{+} cells. **A**, Left panels, CD45+Ter119 and ZsGreen expression in live gated BM cells isolated from *Lepr*-cre^{-}*;Rosa26*$^{+/+}$*;Cxcl12*$^{DsRed/+}$ (left) or from *Lepr*-cre^{+}*;Rosa26*$^{ZsGreen/+}$*;Cxcl12*$^{DsRed/+}$ mice (right). Right panel, CXCL12-DsRed versus ZsGreen expression in gated cells. Approximately 0.58% of ZsGreen^{+} cells are CXCL12-DsRed^{Hi} cells. **B**, Approximately 98% of CXCL12-DsRed^{Hi} cells are marked by *Lepr*-cre recombinase. Left panel indicates CD45+Ter119 and CXCL12-DsRed expression; right panel shows ZsGreen expression in CXCL12-DsRed^{Hi} gated BM cells. Red histogram is *Lepr*-cre^{+}*;Rosa26*$^{ZsGreen/+}$*;Cxcl12*$^{DsRed/+}$. Filled gray histogram is *Lepr*-cre^{-}*;Rosa26*$^{+/+}$*;Cxcl12*$^{DsRed/+}$ control. Data in panels **A** and **B** are representative of 3 independent experiments. **C**, Total number of peripheral blood lymphocytes (left), monocytes (middle), and Granulocytes (right). **D**, Total number of thymic ETPs, **E**, CD4 and CD8 expression in live gated total thymocytes (left, *Lepr*-cre^{-}*;Il7*$^{fl/fl}$; right, *Lepr*-cre^{+}*;Il7*$^{fl/fl}$). **F**, Total number of thymocyte subsets DN, double negative; DP, double positive, CD8 SP, CD8 single positive CD4 SP, CD4 single positive. **G,** TCRβ^{+} cell numbers in peripheral lymph nodes. Where appropriate, *Lepr*-cre^{-}*; Il7*$^{fl/fl}$ mice (black bars) and *Lepr*-cre^{+}*;Il7*$^{fl/fl}$ mice (red bars). Circles depict individual mice analyzed. Data in panels **C-G** are representative of 2 independent experiments. **H**, Flow cytometric analysis of BM from 4-wk-old *Il7*$^{fl/-}$*; Prx1*-cre and control littermate mice. Numbers indicate the percentages of each fraction. **I**, Absolute numbers of indicated cells in the femur and tibia. Bars indicate average, circles depict individual mice. *** $p < 0.001$ (unpaired, two-tailed Student's t-test).

Figure S6, related to Figure 5: **IL-7 expression in NG2^{+} periarteriolar pericytes, and sex-biased *Tie2*-cre activity. A**, 25μm thick femur whole-mount section stained to detect

ECFP expressed by IL-7^{+} cells (green), CD31+CD144 (white), VEGFR3 (red), and SCA-1 (purple) in *Il7*-ECFP mice. Scale bar is 10 μm. **B**, Femur whole-mount section stained to detect ECFP expressed by IL-7^{+} cells (green) and SCA-1 (purple) in *Il7*-ECFP mice. Scale bar is 20 μm. **C**, Flow cytometric analysis of NG2 expression in IL-7^{+} cells. **D,** *Tie2*-cre fate mapping of CD31^{Hi} CD45^{-}Ter119^{-} live BM cells. Right panel, Quantification of *Tie2*-cre fate mapped endothelial cells in BM of male (black) and female (red) mice. Bars indicate average, circles depict individual mice. Data in all panels are representative of 2-3 independent experiments.

Figure S7, related to Figure 7: Specificity of Fgd5-ZsGreen, and comparison of *Lepr*-cre and *Il7*-cre fate mapping of mesenchymal Lepr^{+} BM cells. **A** and **B**, Total BM cells from *Il7*-ECFP mice transplanted with *Fgd5*$^{ZsGreen/+}$ BM cells were stained to detect HSCs and MPPs. **A**, lineage and cKit expression in live gated BM cells (left); cKit and SCA-1 expression in live Lin^{-} cells (middle); FLT3 and CD150 expression in gated LSKs. **B**, Lineage and Fgd5-ZsGreen expression in live gated total BM cells (left); cKit and SCA-1 expression in lineage^{-} ZsGreen^{+} cells (middle); FLT3 and CD150 expression in *Fgd5*-Zsgreen^{+} LSKs (right). **C,** ZsGreen expression driven by *Lepr*-cre in live CD45^{-} Ter119^{-} CD31^{-} CD144^{-} Lepr^{+} BM cells. **D**, tdTomato expression driven by *Il7*-cre in live CD45^{-} Ter119^{-} CD31^{-} CD144^{-} Lepr^{+} BM cells. **E**, Quantification of fate-mapped Lepr^{+} mesenchymal cells: *Lepr*-cre (black), *Il7*-cre (blue). Circles depict individual mice. Data in all panels are representative of 2-3 independent experiments.

Supplementary Experimental Procedures.

Bone marrow chimeras. Adult Boy/J (CD45.1) mice were exposed to two rounds of 6.35 Gy separated by 3h using an XRAD 320 X-ray irradiator (Precision X-ray Inc.). A mixture of $CD45.2^+$ and $CD45.1^+$ (90:10 ratio) bone marrow cells were injected iv. Recipient mice were sacrificed 6 weeks after transplantation and bone marrow, blood, spleen, Peyer's patches, lymph nodes (inguinal, mesenteric, axillary and brachial), thymus, liver and peritoneal cavity were harvested to analyze donor chimerism of Granulocytes, B, T and NK cells. In some cases, recipient mice were bled every 4 weeks between 8-20 weeks to analyze donor chimerism of Granulocytes, B, T and NK cells.

Flow cytometry. Bone marrow cells were isolated by flushing long bones with DMEM (Lonza) containing 2% heat-inactivated fetal bovine serum (FBS), HEPES, PenStrep and L-Glutamine (Cellgro). Red blood cells were lysed with ammonium chloride buffer. Spleen, lymph nodes, Peyer's patch, and thymus were mashed and cells were dissociated to single cell suspension and then filtered through a 70-μm nylon mesh. Liver leukocytes were isolated using Percoll gradients. For flow cytometric analysis of stromal cells, long bones were carefully flushed using HBSS supplemented with 2% of heat-inactivated fetal bovine serum, Penicillin/Streptomycin, HEPES and 200 U/mL Collagenase IV (Worthington Biochemical Corporation). The flushed marrow was then digested at 37ºC for 20 min. Cells clumps were dissociated by gentle pipetting, followed by a further incubation of 10 min at 37ºC. Cells were washed with 5 mL of HBSS/2%FBS, spun at 1000 rpm for 7 min, and stained with the respective antibodies. LEPR stains were done for 1 hour, on ice. All other stains were done for 20 min on ice, followed by 2 washes at 1000 rpm, 7 min. For detection of pSTAT5α, bone marrow cells were flushed from long bones in DMEM containing 0.5% fatty acid free BSA (EMD Biosciences), antibiotics (50IU/mL of ampicillin and 50 mg/mL of streptomycin), and 10 mM HEPES. Cells were fixed in 2% PFA for 10 min at RT and permeabilized with 90% methanol for 30 min and washed with PBS. pSTAT5α was detected using Alexa Fluor 647-conjugated anti-pSTAT5a antibody (BD Pharmigen). Additional antibodies used are listed in table below. Hematopoietic cell populations were identified as follows: HSCs: LIN^- $CD41^-$ $CD48^-$ $cKit^+$ $SCA\text{-}1^+$ $CD150^+$ $FLT3^-$; MPPs: LIN^- $CD41^-$ $CD48^-$ $cKit^+$ $SCA\text{-}1^+$ $CD150^-$ $FLT3^+$; CLPs: LIN^- $IL\text{-}7R^+$ $cKit^{lo}$ $FLT3^+$; CMPs: LIN^- $cKit^+$ $SCA\text{-}1^-$ $CD34^+$ $CD16/32^{lo}$; GMPs:

LIN$^-$ cKit$^+$ SCA-1$^-$ CD34$^+$ CD16/32$^+$; MEPs: LIN$^-$ cKit$^+$ SCA-1$^-$ CD34$^-$ CD16/32$^-$; ETPs: B220$^-$ Gr1$^-$ Ter119$^-$ CD4$^-$ CD8$^-$ cKIT$^+$ CD44$^+$ CD25$^-$; ProB: CD19$^+$ IgM$^-$ IgD$^-$ CD93$^+$ and large forward scatter; PreB: CD19$^+$ IgM$^-$ IgD$^-$ CD93$^+$ and small forward scatter; IgD$^-$ B cells: CD19$^+$ IgM$^+$ IgD$^-$; IgD^{lo} B cells: CD19$^+$ IgM$^+$ IgD^{lo}; Immature B: IgD$^-$ and IgD^{lo} B cells; Mature B: CD19$^+$ IgM$^+$ IgD$^+$; Granulocytes: CD19$^-$ CD3e$^-$ NK1.1$^-$ Gr1$^+$ and large forward and side scatter; NK cells: CD19$^-$ CD3e$^-$ Gr1$^-$ NK1.1$^+$; T cells: Gr1$^-$ NK1.1$^-$ CD19$^-$ CD3e$^+$; B cells: Gr1$^-$ NK1.1$^-$ CD19$^+$ CD3e$^-$. The lineage (LIN) cocktail was as follows: CD19, B220 (excluded in CLP stains), IgD, CD3e, CD4, Gr1, NK1.1, Ter119, CD11b, CD11c.

Table: List of antibodies and fluorochromes used, and vendors.

Antibody	Clone	Conjugation	Source
anti-goat IgG		Alexa Fluor 647	Jackson Immunoresearch
anti-rat IgG		Alexa Fluor 647	Jackson Immunoresearch
anti-rat IgG		Alexa Fluor 555	Life Technologies
anti-rabbit IgG		AMCA	Jackson Immunoresearch
anti-rabbit IgG		Biotin	Jackson Immunoresearch
anti-rabbit IgG	Poly4064	Alexa Fluor 647	Biolegend
CD3e	145-2C11	FITC	Biolegend
CD3e	145-2C11	Pe-Cy7	Biolegend
CD3e	145-2C11	APC	Biolegend
CD3e	145-2C11	Biotin	BD Biosciences
CD4	RM4-5	FITC	Biolegend
CD4	GK1.5	Pe-Cy7	Biolegend
CD8a	53-6.7	Pe-Cy7	Biolegend
CD11c	N418	FITC	Biolegend
CD11c	N418	Biotin	Biolegend

CD11b	M1/70	FITC	Biolegend
CD11b	RM2815	Biotin	Invitrogen
CD16/32	93	PE	Biolegend
CD19	1D3	FITC	Biolegend/ BD Biosciences
CD19	6D5	Pac. Blue	Biolegend
CD19	1D3	PerCP-Cy5.5	eBioscience
CD19	MB19-1	Biotin	eBioscience
CD25	eBio74	Biotin	eBioscience
CD31	390	APC	Biolegend
CD31	390	Alexa Fluor 488	Biolegend
CD34	RAM34	Alexa Fluor 700	BD Biosciences
CD41	MWReg30	FITC	Biolegend
CD44	IM7	PerCP-Cy5.5	Biolegend
CD45R (B220)	RA3-6B2	PerCP-Cy5.5	eBioscience
CD45.1	A20	FITC	Biolegend
CD45.1	A20	PE	BD Biosciences
CD45.1	A20	Pe-Cy7	eBioscience
CD45.1	A20	Pac. Blue	Biolegend
CD45.1	A20	APC-Cy7	Biolegend
CD45.2	104	FITC	BD Biosciences
CD45.2	104	PE	eBioscience
CD45.2	104	Pac. Blue	Biolegend
CD45.2	104	APC-Cy7	Biolegend
CD45.2	104	Alexa Fluor 700	Biolegend
CD48	HM48-1	FITC	Biolegend

CD93 (AA4.1)	AA4.1	APC	eBioscience
CD117 (cKit)	2B8	Biotin	Biolegend
CD117 (cKit)	2B8	APC-H7	BD Biosciences
CD117 (cKit)	2B8	APC	BD Biosciences
CD117 (cKit)	ACK2	BV421	Biolegend
CD117 (cKit)	2B8	BV711	Biolegend
CD127	A7R34	purified	BioXCell
CD127	A7R34	PE	Biolegend
CD127	A7R34	eFlour450	eBioscience
CD135 (Flt3)	A2F10	PE	eBioscience, Biolegend
CD135 (Flt3)	A2F10	Purified	eBioscience
CD140b(PDGFRβ)	APB5	Biotin	Biolegend
CD144	BV13	Alexa Fluor 647	Biolegend
CD150	mShad150	PerCP-eF710	eBioscience
CD150	TC15-12F12.2	PE	Biolegend
CD184 (CXCR4)	2B11	PerCP-eF710	eBioscience
CD184 (CXCR4)		Biotin	BD Biosciences
GFP		purified	Invitrogen
Gr1	RB6-8C5	FITC	Biolegend
Gr1	RB6-8C5	Biotin	eBioscience
IgD	11-26c.2a	PE	Biolegend
IgM	II/41	Pe-Cy7	eBioscience
LepR		Biotin	R&D
Ly6A/E (Sca-1)	D7	Pe-Cy7	eBioscience
Ly6A/E (Sca-1)	E13-161.7	Pe-Cy7	Biolegend

Ly6D	49-H4	FITC/ PE	Biolegend
NK1.1	PK136	FITC	Biolegend
NK1.1	PK136	PerCP-Cy5.5	BD Biosciences
NK1.1	PK136	Biotin	eBioscience
Osteocalcin	mOC(1-20)	purified	Clontech
Osteopontin		purified	R&D
Perilipin	D1D8	purified	Cell Signaling
pSTAT5	47/STAT5(pY694)	Alexa Fluor 647	BD Biosciences
Streptavidin		Alexa Fluor 488	Invitrogen
Streptavidin		PE	BD Biosciences
Streptavidin		PerCP-Cy5.5	BD Biosciences
Streptavidin		Alexa Fluor 647	Invitrogen
Streptavidin		BV 421	Biolegend
Streptavidin		BV 605	Biolegend
Streptavidin		Qdot605	Invitrogen
Ter-119	Ter119	FITC	Biolegend
Ter-119	Ter119	Biotin	BD Biosciences
Ter-119	Ter119	PE	Biolegend
Ter-119	Ter119	APC-Cy7	Biolegend

Immunostaining of bone marrow.

BM Sections. Freshly dissected long bones were fixed in a paraformaldehyde-based fixative at 4ºC for 6h or overnight. Then, bones were washed in PBS 1x, for 10 min, 3 times. Bones were dehydrated in a solution of 30% sucrose in PBS, at 4ºC overnight. Samples were embedded in OCT and snap-frozen in an ethanol/dry ice bath. Frozen

sections were prepared according to the Kawamoto method or using the CryoJane tape transfer system (Leica). 7-µm or 25-µm sections were dried overnight. Sections were rehydrated in PBS with 0.05% BSA for 10 min before staining. Goat serum (4%) in PBS with 1%BSA was used for blocking. Primary antibodies were applied to the slides for 3h at room temperature or overnight at 4ºC, and this was followed by secondary antibody incubation by for 1-2h at room temperature with repeated washes in between. Primary antibodies were rabbit anti-GFP (Invitrogen), rat anti-IL7Ra (BioXCell). Sections were incubated with a blocking solution of rat serum (4%), BSA (1%) in PBS at least for 30 min. Then, fluorochrome-conjugated antibodies were applied to the slides for at least 1h. Slides were mounted with Fluormount-G (SourthernBiotech) or with a 30% glycerin solution. Images were acquired on a Zeiss Z1 Observer fluorescent microscope equipped with Colibri LED light sources, or on a Leica SP8 confocal microscope.

Femur Whole Mounts. Freshly dissected long bones were fixed and frozen as described above except when staining for direct visualization of HSCs and MPPs. For this purpose, mice were anesthetized with ketamine/xylazine and fixed in vivo by perfusion with paraformaldehyde (final concentration of 2%). Lone bones were removed and fixed ex vivo for a further 4 h followed by washing and dehydration in sucrose as described above. To obtain whole mounts, bones were shaved on both sides using a cryostat until the bone marrow cavity was fully exposed. The bones were then brought to RT to allow the OCT to melt, and then washed briefly with 1x PBS. Whole mounts were permeabilized with 0.05% Triton-X in 1x PBS for 5h and then blocked overnight with donkey serum (4%) and/or goat serum (4%) at 4°C. Whole mounts were stained with primary antibodies for 2-3 days at 4°C and secondary antibodies for 1 day at 4°C. HSCs were identified as LIN^- $CD41^-$ $CD48^-$ $CD150^+$ (and $cKit^+$ where applicable) cells. MPPs were identified as LIN^- $CD41^-$ $CD48^-$ $CD150^+$ $cKit^+$ cells. Images were analyzed using the Imaris software. For random positioning analysis, spots were placed on all $DAPI^+$ or LIN^+ cells, and each spot was given a unique identification number ranging from 0 to N. The randbetween() function in Microsoft Excel was then used to generate random numbers between 0 and N (each of which corresponded to a $DAPI^+$ or LIN^+ spot). The distance between the selected spot and the closest IL-7^+ cell was then measured using the measurement function in Imaris. Between 10-20 random spots were placed in each field-

of-view in which an HSC or MPP was found, giving a total of over 1000 random spots. Random spots were 8 μm in diameter, corresponding to the average diameter of HSCs and MPPs that we observed. For detection of Fgd5-ZsGreen^{+} HSCs, Il7-ECF reporter mice were exposed to two rounds of 5.5 Gy separated by 3h, and reconstituted with 3x10^{6} BM cells from Fgd5$^{ZsGreen/+}$ mice. Mice were sacrificed > 8 weeks post-transplant. Images were acquired on a Leica SP8 confocal microscope.

Chemotaxis assay. Total bone marrow cells were stained against lineage markers, and incubated with Dynabeads (Sigma). A Lin^{-} cell enriched cell suspension was recovered and incubated for 30 min at 37ºC in DMEM containing 0.5% fatty acid free BSA (EMD Biosciences), antibiotics (50IU/mL of ampicillin and 50 mg/mL of streptomycin; Cellgro), and 10 mM HEPES (Cellgro). Cells were allowed to migrate through 5μm-pore sized transwells (Corning) for 3h at 37ºC with 5% CO_2. Cells were collected, stained, resuspended in 45μL of staining buffer and analyzed by flow cytometry for 45s.

CLP single cell differentiation in vitro assays. OP9 stromal cells were seeded in 96-well plates (2,500 cells/well), left overnight at 37 ºC, and irradiated (10 Gy) before plating of hematopoietic cells. Single live Ly6D^{+} CLP (Lin^{-} CD127^{+} cKIT^{int} SCA1^{+} FLT3^{+} Ly6D^{+}) were sorted to each well. RPMI (containing 10%FBS, HEPES, PenStrep, L-Glutamine, and 50μM β-Mercaptoethanol) supplemented with saturating amounts of Flt3L, SCF, IL-7 and IL-2 (final concentration of 50 ng/mL) was added to each well. On day 5, culture medium was replaced with RPMI supplemented with FLT3L and IL-7 only. On day 10, scores were assigned for B cells (CD19^{+}), myeloid (CD11b^{+}), NK cells (NK1.1^{+}) and dendritic cells (CD11c^{+}) by flow cytometry.

Mesenchymal progenitor cell sorting, and mSCF analyses. Long bones recovered from 3-4 C57BL/6 mice (ages 6-12 week) were flushed and digested with Collagenase as described above. Cells were counted and stained with biotinylated anti-LEPR antibody for 1 hour, on ice. After washing, cells were incubated with anti-CD45, anti-Ter119, anti-CD31, anti-CD144 antibodies, Streptavidin BV605, and DAPI for 30 min. Fresh unfixed collagenase-digested BM cells were stained with rat anti-mouse SCF antibody (R&D) for 1 hour. SCF staining was revealed with an anti-rat AlexaFluor 555 antibody. Cells were then blocked with rat serum (4%), and stained for the remaining surface markers (CD45, Ter119, CD31, CD144). Cells were acquired and sorted on a BD FACS Aria II equipped

with UV (355nm), Violet (405nm), Blue (488nm), Green (532nm) and Red (640nm) lasers, and with FACSDiva 7.

Adipocyte isolation. Bone marrow from long bones was gently flushed with HBSS/2% FBS supplemented with 200U/mL Collagenase IV and DNase I (Roche), and incubated at 37ºC for 15 min. After digestion, the cell suspension was washed and allowed to stand for 10 min, and then spun at 500*g* for 5 min. Adipocyte layers were gently recovered from the upper phase.

In vitro osteoblast differentiation. Neonatal calvaria osteoblasts were prepared from 3-5-day old C57BL/6 mice. Briefly, calvariae were treated with 4mM EDTA in PBS and sequentially digested with collagenase (200 U/mL) for 5 times. Fractions 3-5 were plated in α-MEM containing 10% FBS, 10 mM HEPES, and Penn/Strep. Media was changed every 3-4 days. Adult osteoblasts were differentiated as follows: BM cells flushed from femurs and the bones were incubated in DMEM containing 2% Collagenase A (Roche) and antibiotics at 37°C with agitation for 30 min (500 µl/bone), then washed with DMEM. Bones were incubated a second time in DMEM containing 2% Collagenase A and antibiotics at 37°C with agitation for 30 min and this fraction was collected. To remove contaminating hematopoietic cells, cells were incubated with biotin anti-CD45 (Clone 30-F11, Biolegend) at 1:100 dilution in 100 µl of DMEM containing 2% FBS for 20 min at 4°C then washed two times, cells were then resuspended in 100 µl of DMEM containing 10 µL strepavidin beads (Invitrogen) and incubated at 4°C with gentle shaking for 45 min. Labeled hematopoietic cells were separated using a magnetic field and remaining cells were plated up to $1x10^6$ per well on 6 well plates in α-MEM containing 10% FBS, 10 mM HEPES, 50 µg/mL L-ascorbic acid 2-phosphate (Sigma), 10 nM glycerophosphate (Sigma), and 10 nM dexamethasone (Sigma). Media was changed every 3-4 days.

RNA isolation and Quantitative Real Time PCR. Total RNA was isolated from sorted stromal cells, adipocytes and cultured osteoblasts using mRNeasy kit (Qiagen). cDNA was synthetized from the isolated RNA and Q-PCR was the SensiFAST™ SYBR Lo-ROX Kit (Bioline) and the CFX Touch™ Real-Time PCR detection system (BioRad). *Hprt* mRNA levels were used control. Murine PCR primer sequences: *Bglap*-Fw, GGG CAA TAA GGT AGT GAA CAG; *Bglap*-Rv, GCA GCA CAG GTC CTA AAT AGT;

Adipoq-Fw, TGT TCC TCT TAA TCC TGC CCA; *Adipoq*-Rv, CCA ACC TGC ACA AGT TCC CTT; *Plin1*-Fw, CAT CTC TAC CCG CCT TCG AA; *Plin1*-Rv, TGC TTG CAA TGG GCA CAC T; *Il7*-Fw, GCT GCC TGT CAC ATC ATC TG; or GCC ACA TTA AAG ACA AAG AAG GT (for gene deletion); *Il7*-Rv, CAG CAC GAT TTA GAA AAG CAG C or TGG TTC ATT ATT CGG GCA AT (for gene deletion); *Cxcl12*-Fw, CGC CAA GGT CGT CGC CG; *Cxcl12*-Rv, TTG GCT CTG GCG ATGT GGC; *Hprt* -Fw, AGG TTG CAA GCT TGC TGG T; *Hprt* -Rv, TGA AGT ACT GAT TAT AGT CAA GGG CA.