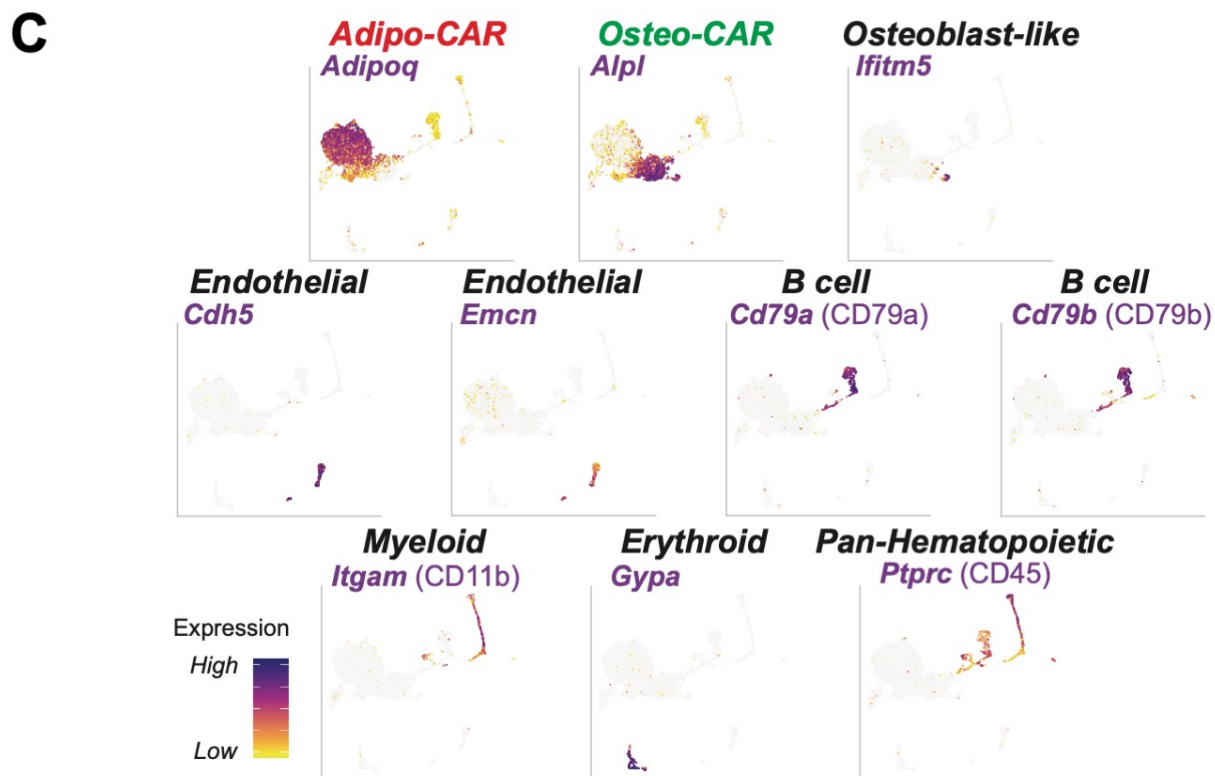
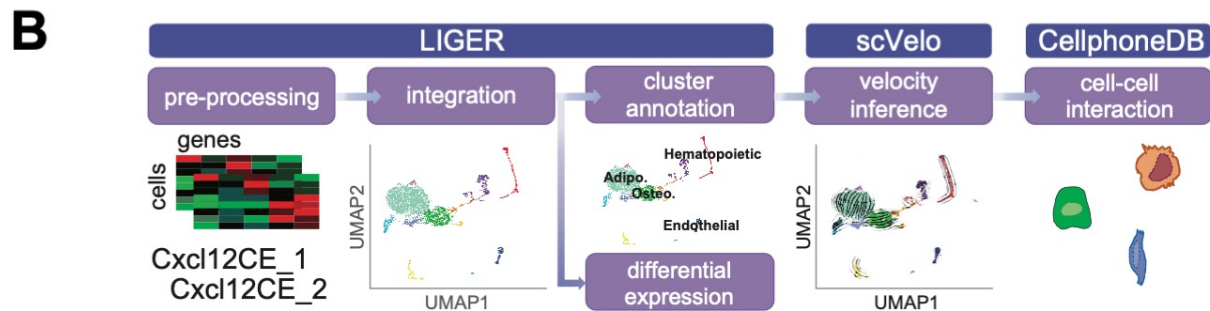
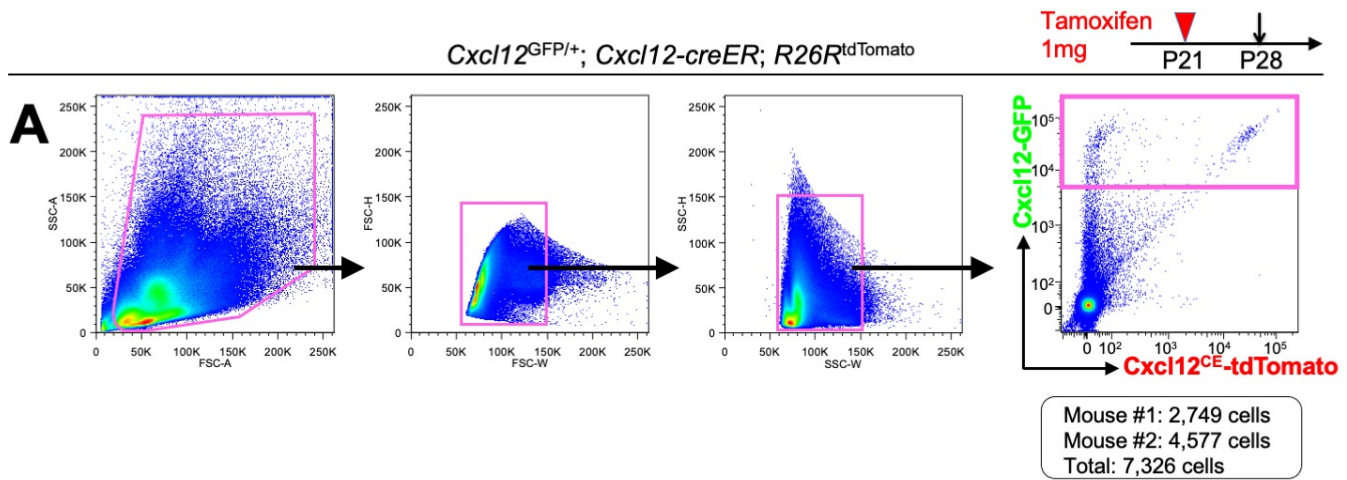


Figure S1



1 **Figure S1. Single cell RNA-seq analysis of Cxcl12-GFP⁺ bone marrow cells.**

2

3 (A) FACS-sorting strategy for Cxcl12-GFP⁺ cells (red box), cells isolated from *Cxcl12*^{GFP/+};
4 *Cxcl12-creER*; *R26R*^{tdTomato} femurs for single cell RNA-seq analysis.

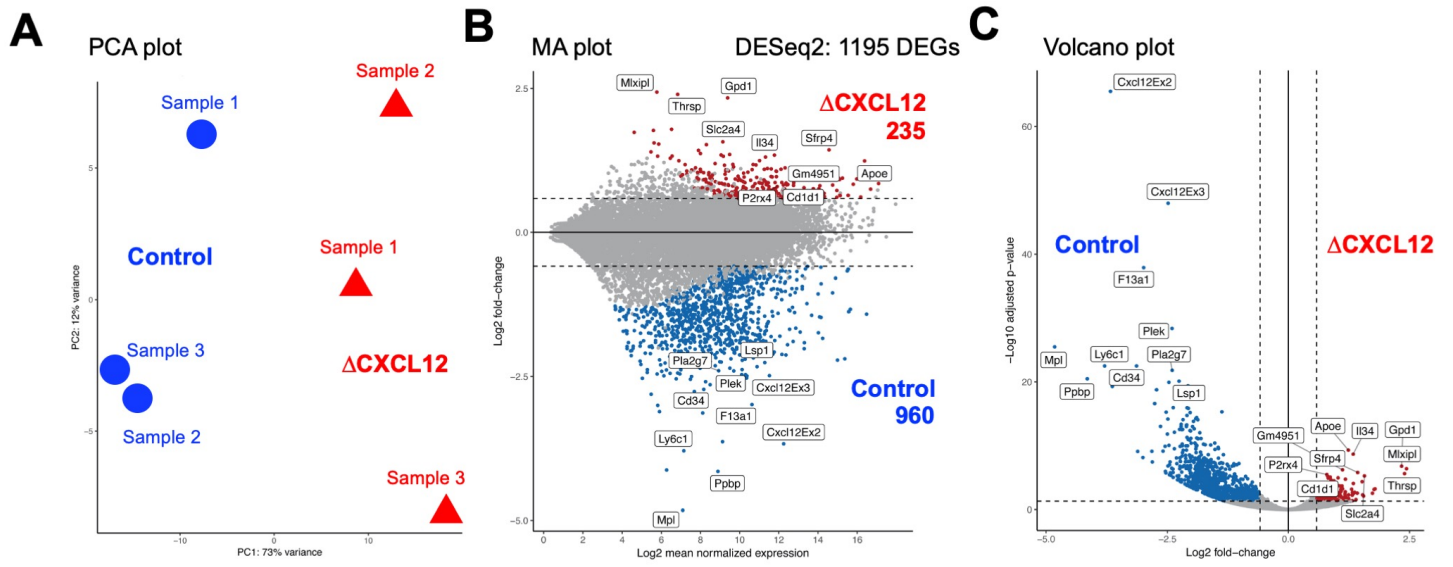
5 (B) Overview of the single-cell RNA-seq analysis workflow. LIGER takes two single-cell
6 datasets, Cxcl12CE_1 and Cxcl12CE_2 as input, integrates the single-cell transcriptome data,
7 and determines clustering assignments. ScVelo infers the future state of individual cells.

8 CellphoneDB infers relevant receptor-ligand interactions between cell populations.

9 (C,D) UMAP-based visualization of major classes of FACS-sorted cells. Feature plots of cell-
10 type specific markers. Purple: high expression. *n*=7,326 cells merged from two biological
11 replicates.

12

Figure S2



13 **Figure S2. Comparative RNA-seq analysis of CXCL12-deficient Cxcl12-GFP⁺ stromal cells.**

14

15 RNA-seq analysis of Δ CXCL12 cells. **(A)** Principal component analysis (PCA) plot of six
16 biological samples. Triangles: Δ CXCL12 (cKO, red), circles: Control (blue). *x*-axis: PC1, 73%
17 variance, *y*-axis: PC2, 12% variance. *n*=3 biological replicates per group. **(B)** MA plot, *x* axis:
18 \log_2 FPKM, *y* axis: \log_2 fold-change. *y* > 0.58 represents genes enriched in Δ CXCL12 (cKO, red),
19 *y* < -0.58 represents genes enriched in Control (blue). **(C)** Volcano plot, *x* axis: \log_2 fold-change,
20 *y* axis: $-\log_{10}$ adjusted *p*-value. *x* > 0.58 represents genes enriched in Δ CXCL12 (cKO, red), *x* < -
21 0.58 represents genes enriched in Control (blue). Each dot represents a gene.

22

Supplemental Table 1

A Pathway analysis (KEGG), $p < 0.001$ (FDR)

KEGG pathway Name	pv_fdr
Hematopoietic cell lineage	1.73E-16
Phagosome	1.69E-08
Chemokine signaling pathway	8.04E-07
Asthma	8.04E-07
Cell adhesion molecules (CAMs)	1.65573E-06
Natural killer cell mediated cytotoxicity	9.11471E-06
Tuberculosis	1.79698E-05
Fc gamma R-mediated phagocytosis	3.32761E-05
Leukocyte transendothelial migration	3.59835E-05
Regulation of actin cytoskeleton	4.62885E-05
Staphylococcus aureus infection	4.62885E-05
Primary immunodeficiency	7.88074E-05
Cytokine-cytokine receptor interaction	8.847E-05
Intestinal immune network for IgA production	8.847E-05
Platelet activation	8.847E-05
Leishmaniasis	0.000116814
Viral myocarditis	0.000178236
B cell receptor signaling pathway	0.000178236
Amoebiasis	0.00022976
Osteoclast differentiation	0.000300852
Inflammatory bowel disease (IBD)	0.001060829
Fluid shear stress and atherosclerosis	0.001060829
Neuroactive ligand-receptor interaction	0.001063463
NF-kappa B signaling pathway	0.001485707
Jak-STAT signaling pathway	0.003433564
Rap1 signaling pathway	0.005295024
Systemic lupus erythematosus	0.005524801
Fc epsilon RI signaling pathway	0.007246951

B Gene Ontology analysis – Biological Process, $p < 0.001$ (Elim)

GO ID	GO Name	countDE	countAll	pv_elim
GO:0045087	innate immune response	113	472	3.50E-16
GO:0006954	inflammatory response	111	468	1.10E-13
GO:0030593	neutrophil chemotaxis	33	68	2.00E-12
GO:0002250	adaptive immune response	82	282	5.20E-09
GO:0019886	antigen processing and presentation of exogenous peptide antigen via MHC class II	10	14	1.30E-08
GO:0006909	phagocytosis	53	155	2.10E-08
GO:0006955	immune response	229	917	2.40E-08
GO:0042742	defense response to bacterium	44	133	2.90E-08
GO:0050853	B cell receptor signaling pathway	23	37	1.20E-07
GO:0045766	positive regulation of angiogenesis	29	121	2.00E-07
GO:0002548	monocyte chemotaxis	16	34	2.30E-07
GO:0042832	defense response to protozoan	12	25	2.30E-07
GO:0072672	neutrophil extravasation	9	13	3.60E-07
GO:0090023	positive regulation of neutrophil chemotaxis	12	26	4.00E-07
GO:0008360	regulation of cell shape	28	122	8.30E-07
GO:0006911	phagocytosis, engulfment	16	40	0.0000012
GO:0070098	chemokine-mediated signaling pathway	13	33	0.0000012
GO:0007229	integrin-mediated signaling pathway	21	78	0.0000013
GO:0007186	G-protein coupled receptor signaling pathway	72	428	0.0000025
GO:0033031	positive regulation of neutrophil apoptotic process	5	5	0.0000043
GO:0038083	peptidyl-tyrosine autophosphorylation	15	50	0.0000099
GO:0050830	defense response to Gram-positive bacterium	17	54	0.000016
GO:0035458	cellular response to interferon-beta	12	35	0.000017
GO:0030889	negative regulation of B cell proliferation	8	16	0.000018
GO:0042098	T cell proliferation	43	164	0.000022
GO:0050829	defense response to Gram-negative bacterium	14	37	0.000036
GO:0070527	platelet aggregation	18	51	0.000049
GO:0050859	negative regulation of B cell receptor signaling pathway	4	4	0.000051
GO:0050766	positive regulation of phagocytosis	14	51	0.000058
GO:0071346	cellular response to interferon-gamma	14	51	0.000058
GO:0032930	positive regulation of superoxide anion generation	7	14	0.000061
GO:0030890	positive regulation of B cell proliferation	11	34	0.00007
GO:0050861	positive regulation of B cell receptor signaling pathway	5	7	0.000078
GO:0032496	response to lipopolysaccharide	41	213	0.000085
GO:0090280	positive regulation of calcium ion import	6	11	0.00012
GO:0019233	sensory perception of pain	18	81	0.00012
GO:0045730	respiratory burst	8	20	0.00013
GO:0002523	leukocyte migration involved in inflammatory response	7	16	0.00018
GO:0042100	B cell proliferation	30	69	0.00019
GO:0019370	leukotriene biosynthetic process	6	12	0.00021
GO:0032743	positive regulation of interleukin-2 production	11	27	0.00027
GO:0046641	positive regulation of alpha-beta T cell proliferation	7	17	0.00028
GO:0045577	regulation of B cell differentiation	12	29	0.00036
GO:0042119	neutrophil activation	17	23	0.00039
GO:0043552	positive regulation of phosphatidylinositol 3-kinase activity	8	23	0.0004
GO:2001214	positive regulation of vasculogenesis	5	9	0.00041
GO:0061844	antimicrobial humoral immune response mediated by antimicrobial peptide	7	18	0.00042
GO:0014068	positive regulation of phosphatidylinositol 3-kinase signaling	13	54	0.00044
GO:0070374	positive regulation of ERK1 and ERK2 cascade	25	146	0.00052
GO:0002040	sprouting angiogenesis	15	69	0.00054
GO:0043312	neutrophil degranulation	9	11	0.0006
GO:0038094	Fc-gamma receptor signaling pathway	5	7	0.0006
GO:0001782	B cell homeostasis	10	36	0.0006
GO:0002337	B-1a B cell differentiation	3	3	0.0006
GO:0002554	serotonin secretion by platelet	3	3	0.0006
GO:0097029	mature conventional dendritic cell differentiation	3	3	0.0006
GO:0002606	positive regulation of dendritic cell antigen processing and presentation	4	6	0.00067
GO:0007596	blood coagulation	40	146	0.00073
GO:0033198	response to ATP	8	25	0.00075
GO:0006968	cellular defense response	5	10	0.00075
GO:0048260	positive regulation of receptor-mediated endocytosis	12	51	0.00091
GO:0019731	antibacterial humoral response	6	15	0.00093
GO:0044130	negative regulation of growth of symbiont in host	6	15	0.00093

23 **Supplemental Table 1, related to Figure 4. Functional CXCL12 maintains reticular stromal**
24 **cells' physical interactions with hematopoietic cells.**

25

26 Enriched terms for Pathway analysis (KEGG, A) and Gene Ontology analysis – Biological
27 Process (B), based on differentially expressed genes between Control and Δ CXCL12 cells.

28