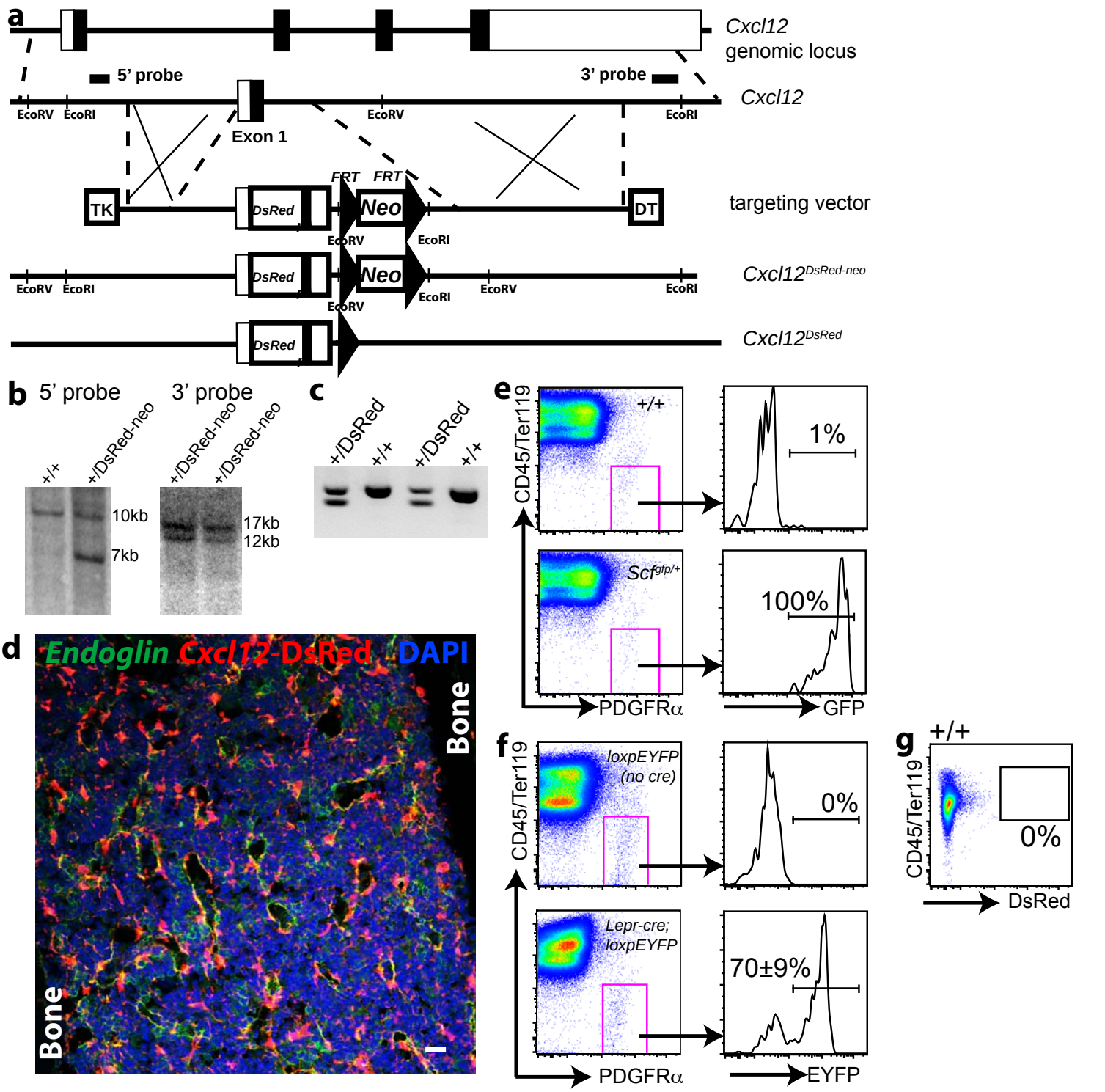
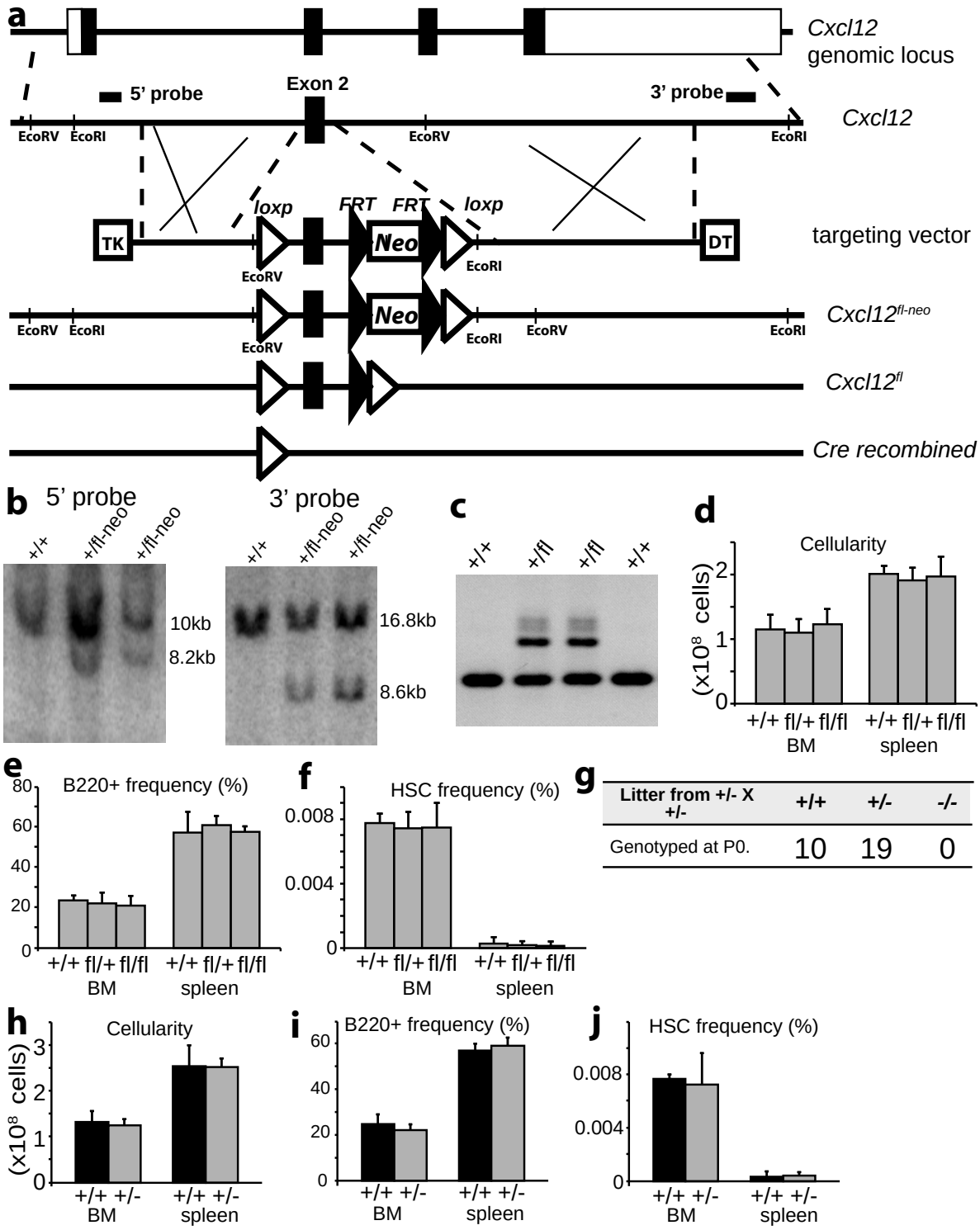
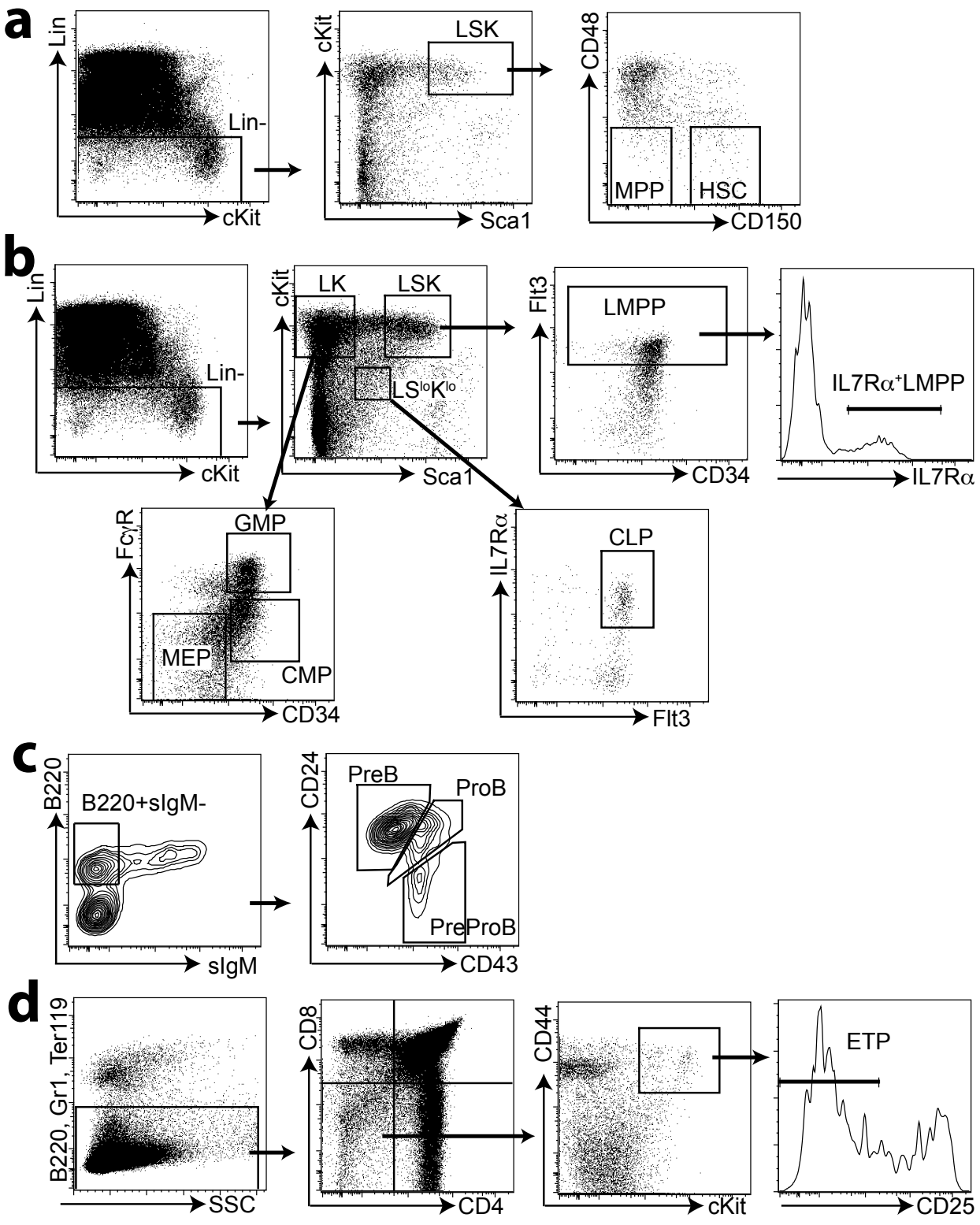


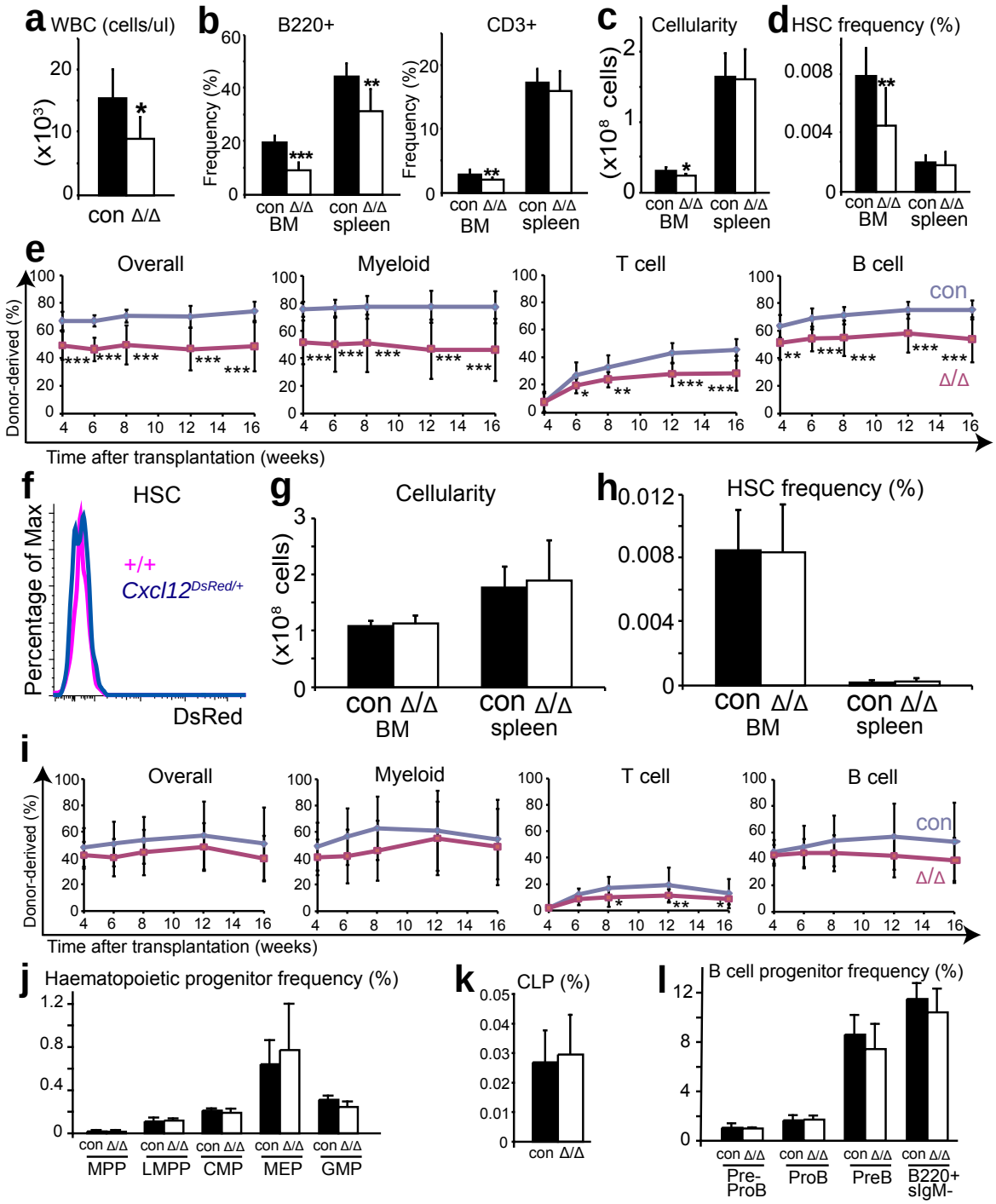
Supplementary Figure 1. The generation and analysis of *Cxcl12*^{DsRed} mice demonstrated that *Cxcl12* is primarily expressed by endothelial cells and perivascular stromal cells in the bone marrow. a, The targeting vector to generate the *Cxcl12*^{DsRed} allele was generated by recombineering[31]. The vector was modified by inserting a *DsRedE2-polyA-Frt-Neo-Frt* cassette into the second exon of *Cxcl12*. **b**, The targeting vector was electroporated into C57BL-derived Bruce4 ES cells. Correctly targeted ES cell clones were identified by Southern blotting using 5' and 3' probes and used for generating chimeric mice. Genomic DNA was digested with EcoRV for the 5' probe and EcoRI for the 3' probe. Chimeric mice were bred with C57BL/6 mice to get germline transmission. The *Neo* cassette was subsequently deleted by mating these mice with *Flpe* mice[32]. **c**, PCR genotyping demonstrated germline transmission of the *Cxcl12*^{DsRed} allele. **d**, A low magnification immunofluorescence image of a bone marrow section from a *Cxcl12*$^{DsRed/+}$ mouse showing that DsRed (stained with an anti-RFP antibody, red) was primarily expressed by perivascular stromal cells and endothelial cells throughout the bone marrow. Bone marrow endothelial cells were stained with an anti-Endoglin antibody (green). Nuclei were stained with DAPI (blue). **e**, Flow cytometric analysis of bone marrow cells from *Scf*$^{gfp/+}$ mice showing that essentially all CD45/Ter119$^-$PDGFRα$^+$ stromal cells were *Scf*-GFP$^+$. **f**, 70±9% of CD45/Ter119$^-$PDGFRα$^+$ stromal cells were EYFP$^+$ in *Lepr-cre; loxpEYFP*$^+$ mice (n=4). **g**, Control for Fig. 1n showing CD45/Ter119$^+$ haematopoietic cells from control bone marrow did not express DsRed. Scale bar, 20um.



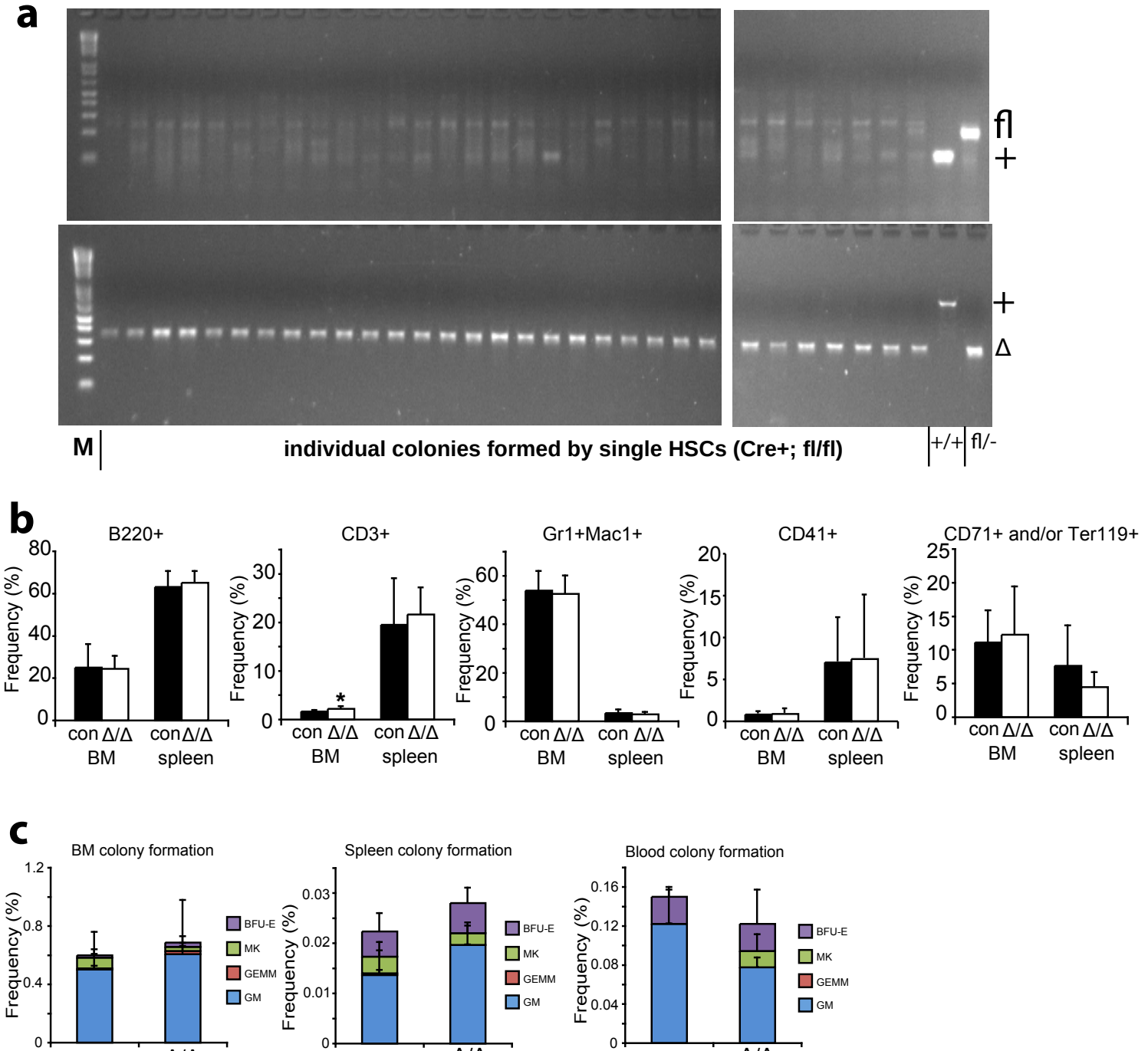
Supplementary Figure 2. The generation and characterization of *Cxcl12*^{fl} mice. a, The targeting vector was constructed by recombineering[31] in which a loxp site was inserted 5' of exon2 and an *Frt-Neo-Frt-loxp* cassette was inserted 3' of exon2. Sequence conservation among species was assessed to avoid disrupting intronic regulatory elements. Upon Cre mediated DNA recombination, exon2 is excised, leading to a frame-shift and loss of *Cxcl12* function. **b**, Southern blotting identified the correctly targeted Bruce4 ES cell clones used to generate chimeric mice. Genomic DNA was digested with EcoRV for the 5' probe and EcoRI for the 3' probe. Chimeric mice were bred with C57BL/6 mice to obtain germline transmission. The *Neo* cassette was subsequently deleted by mating with *Flpe* mice[32]. **c**, PCR genotyping indicated germline transmission of the *Cxcl12*^{fl} allele. **d-f**, *Cxcl12*$^{fl/+}$ and *Cxcl12*$^{fl/fl}$ mice that lacked Cre recombinase appeared to retain wild-type *Cxcl12* function as they had normal cellularity, B220^{+} B cell frequencies, and HSC frequencies in the bone marrow and spleen (n=4-8). **g**, A predicted null allele of *Cxcl12* (*Cxcl12*$^{-}$) was generated by mating *Cxcl12*$^{fl/+}$ mice with *CMV-cre* mice. The progeny from intercrossing *Cxcl12*$^{+/-}$ mice revealed that *Cxcl12*$^{-/-}$ pups died before birth but that *Cxcl12*$^{+/-}$ progeny were generated in expected numbers. **h-j**, *Cxcl12*$^{+/-}$ mice had normal cellularity, B220^{+} B cell frequencies, and HSC frequencies in the bone marrow and spleen (n=3-5). +, wild-type *Cxcl12* allele; -, germline deleted allele; fl, *Cxcl12*^{fl} allele. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance.



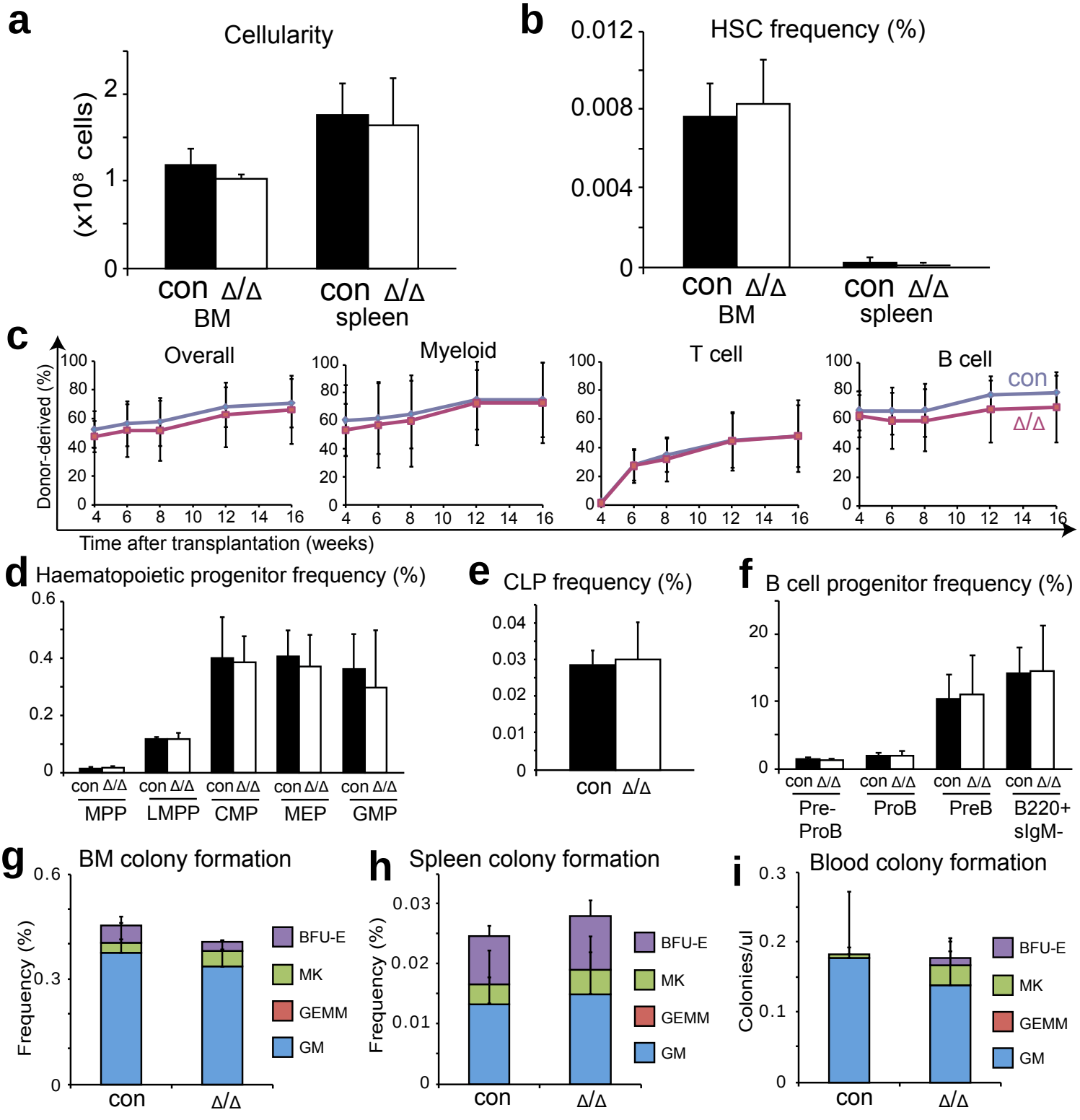
Supplementary Figure 3. Representative flow cytometry gates used to isolate the stem and progenitor cell populations characterized in this study. a, CD150^{+}CD48^{-}Lineage^{-}Sca1^{+}cKit^{+} HSCs[4] and CD150^{-}CD48^{-}LSK multipotent progenitors (MPPs[33]), **b**, Flt3^{+}LSK lymphoid-primed MPPs (LMPPs[24]), IL7Rα^{+}LMPPs, CD34^{+}FcγR^{-}Lineage^{-}Sca1^{-}cKit^{+} common myeloid progenitors (CMPs[2]), Lineage^{-}Sca1^{low}cKit^{low}Flt3^{+}IL7Rα^{+} common lymphoid progenitors (CLPs[3,34]), CD34^{-}FcγR^{-}Lineage^{-}Sca1^{-}cKit^{+} megakaryocytic/erythroid progenitors (MEPs[2]), and CD34^{+}FcγR^{+}Lineage^{-}Sca1^{-}cKit^{+} granulocyte/macrophage progenitors (GMPs[2]). **c**, B220^{+}sIgM^{-}CD43^{+}CD24^{-} Pre-Pro B cells, B220^{+}sIgM^{-}CD43^{+}CD24^{+} Pro B cells, B220^{+}sIgM^{-}CD43^{-} Pre B cells, and B220^{+}sIgM^{-} B cells[35]. **d**, B220^{-}Gr1^{-}Ter119^{-}CD4^{-}CD8^{-}CD44^{+}cKit^{+}CD25^{-} ETPs[36].



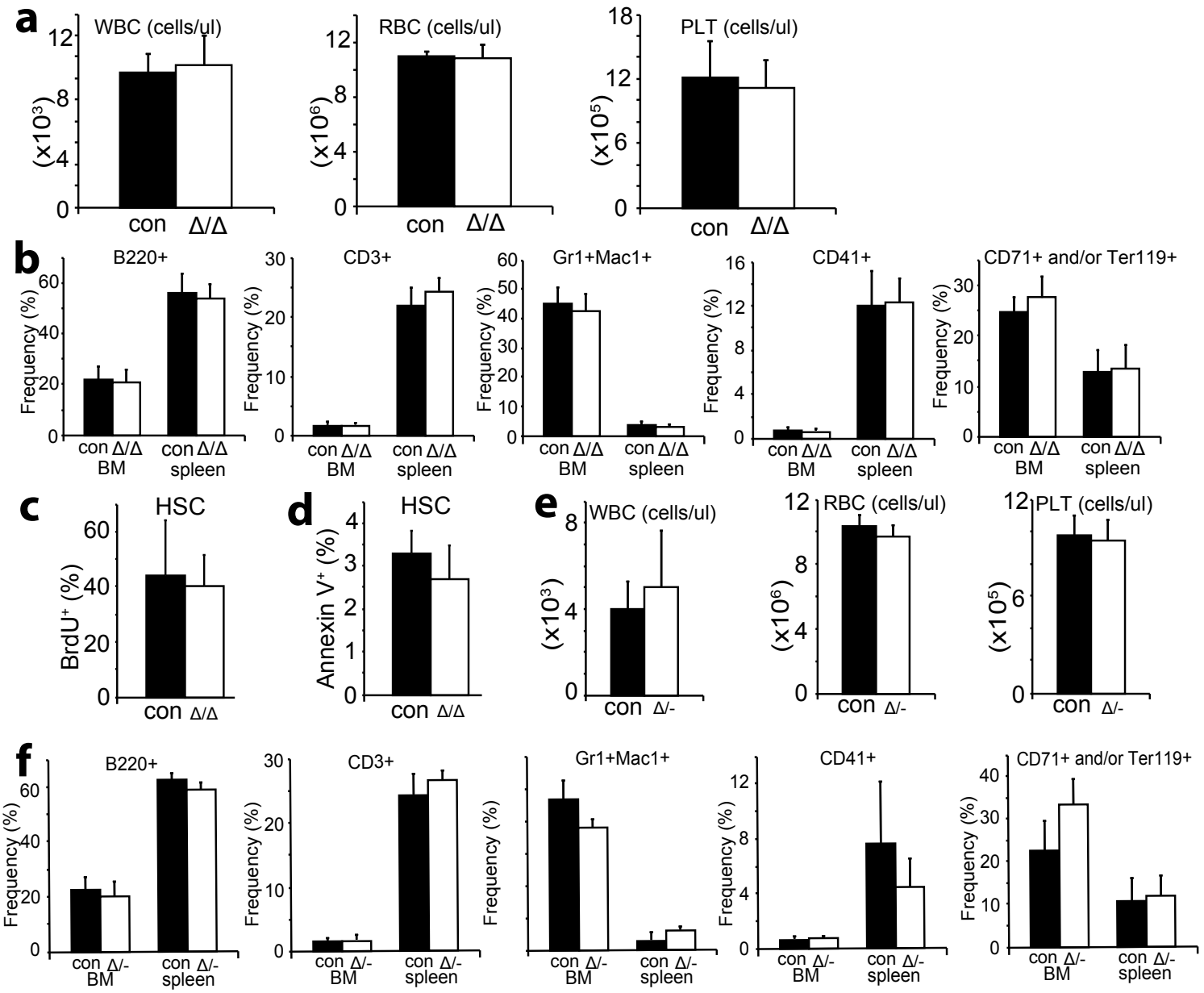
Supplementary Figure 4. *Cxcl12* is required non-cell-autonomously for adult HSC maintenance and lymphopoiesis. a-d, Global deletion of *Cxcl12* in adult *Ubc-creER; Cxcl12$^{fl/fl}$* mice reduced white blood cell counts (n=4-5) (**a**), the frequency of B220^{+} B cells and CD3^{+} T cells (n=12-14) (**b**), bone marrow cellularity (two femurs and two tibias) (**c**), and HSC frequency in the bone marrow (**d**) relative to littermate controls (n=6-8). **e**, 3x10^{5} donor bone marrow cells from *Ubc-creER; Cxcl12$^{fl/fl}$* adult mice were competitively transplanted with recipient bone marrow cells into irradiated recipient mice. The percentages of donor-derived Mac-1^{+} myeloid, CD3^{+} T, and B220^{+} B cells in the blood were analyzed for 16 weeks after transplantation (three experiments with a total of 14-15 recipients per genotype). **f**, HSCs did not express *Cxcl12*-DsRed by flow cytometry. **g**, **h**, Deletion of *Cxcl12* from haematopoietic cells using *Vav1-cre* did not significantly affect bone marrow or spleen cellularity or HSC frequency (n=6). **i**, 3x10^{5} donor bone marrow cells from *Vav1-cre; Cxcl12$^{fl/fl}$* mice were transplanted with recipient bone marrow cells into irradiated recipient mice and the percentages of donor-derived blood cells were analyzed for 16 weeks after transplantation (three experiments with a total of 13-14 recipients per genotype). **j-l**, *Vav1-cre; Cxcl12$^{fl/fl}$* mice had normal frequencies of MPPs, LMPPs, CMPs, MEPs, GMPs (**j**), CLPs (**k**), and committed B lineage progenitors in their bone marrow (**l**) (n=3). MPP, multipotent progenitor; LMPP, lymphoid-primed MPP; CMP, common myeloid progenitor; MEP, megakaryocytic/erythroid progenitor; GMP, granulocyte/macrophage progenitor; CLP, common lymphoid progenitor. Δ, recombined *Cxcl12^{fl}* allele; con, negative control mice with the following *Cxcl12* genotypes: +/+ or fl/+ or fl/fl without *cre*. *Cxcl12$^{fl/+}$* and *Cxcl12$^{fl/fl}$* mice lacking cre had phenotypes indistinguishable from *Cxcl12$^{+/+}$* mice (Supplementary Fig. 2d-f), and therefore were pooled as negative controls. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance. *P<0.05, **P<0.01, ***P<0.001.



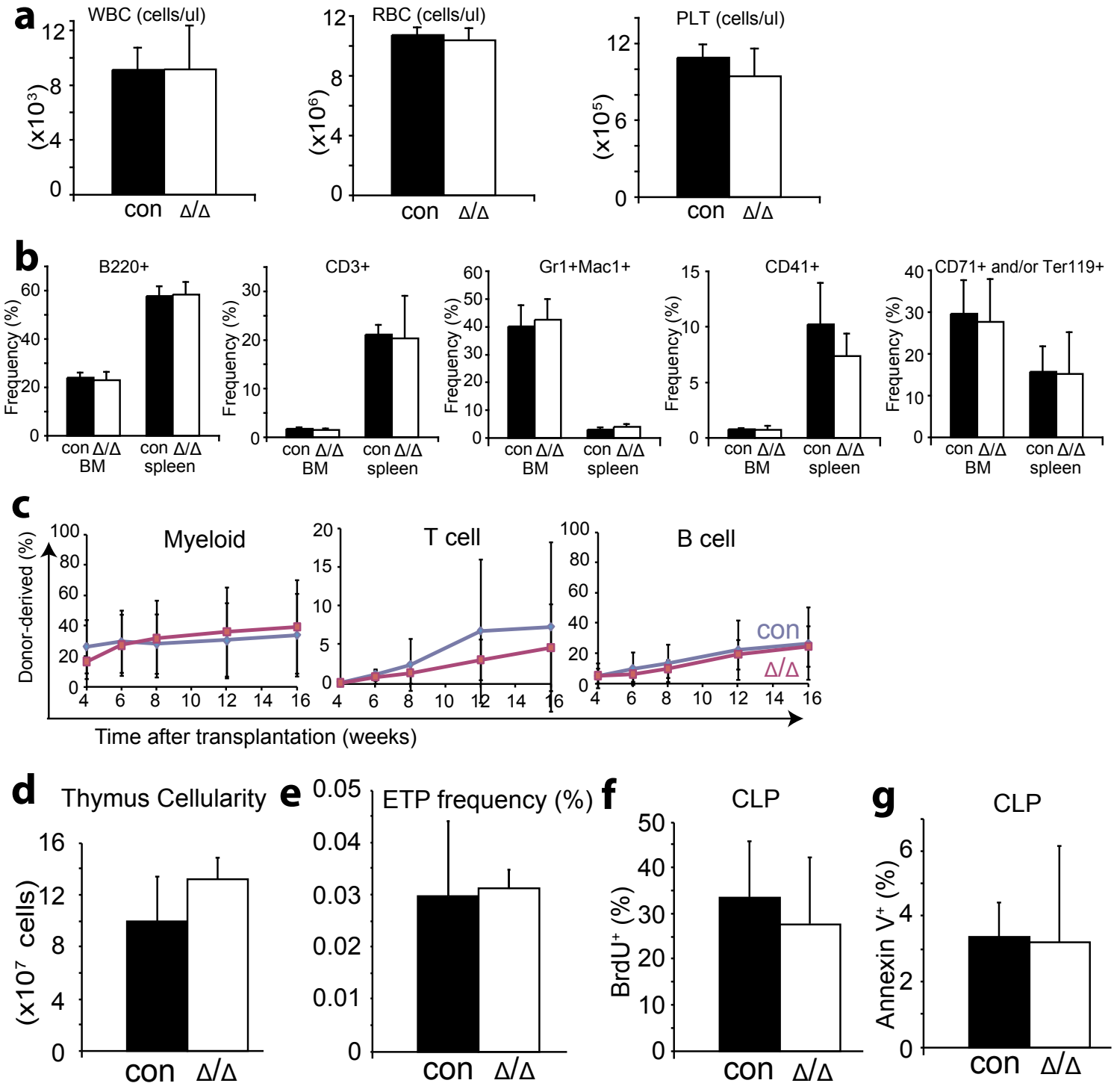
Supplementary Figure 5. Analysis of *Vav1-cre; Cxcl12$^{fl/fl}$* mice revealed that the *Cxcl12^{fl}* allele is efficiently recombined and CXCL12 production by haematopoietic cells is not required for the development or maintenance of adult haematopoiesis. a, Genotyping of colonies formed by individual HSCs isolated from adult *Vav1-cre; Cxcl12$^{fl/fl}$* mice . Vav1-Cre recombined both *Cxcl12^{fl}* alleles in all 31 colonies analyzed from two independent experiments. **b**, Adult *Vav1-cre; Cxcl12$^{fl/fl}$* mice exhibited normal frequencies of B cells, T cells, myeloid cells, megakaryocyte lineage cells, and erythroid lineage cells in the bone marrow except a small but significant increase in the frequency of CD3^{+} T cells in the bone marrow (n=6). **c**, Adult *Vav1-cre; Cxcl12$^{fl/fl}$* mice had normal frequencies of myeloerythroid colony-forming progenitors in the bone marrow, spleen, and blood (n=3-5). Δ, recombined *Cxcl12^{fl}* allele; con, negative control mice with the following *Cxcl12* genotypes: +/+ or fl/+ or fl/fl without cre. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance. *P<0.05.



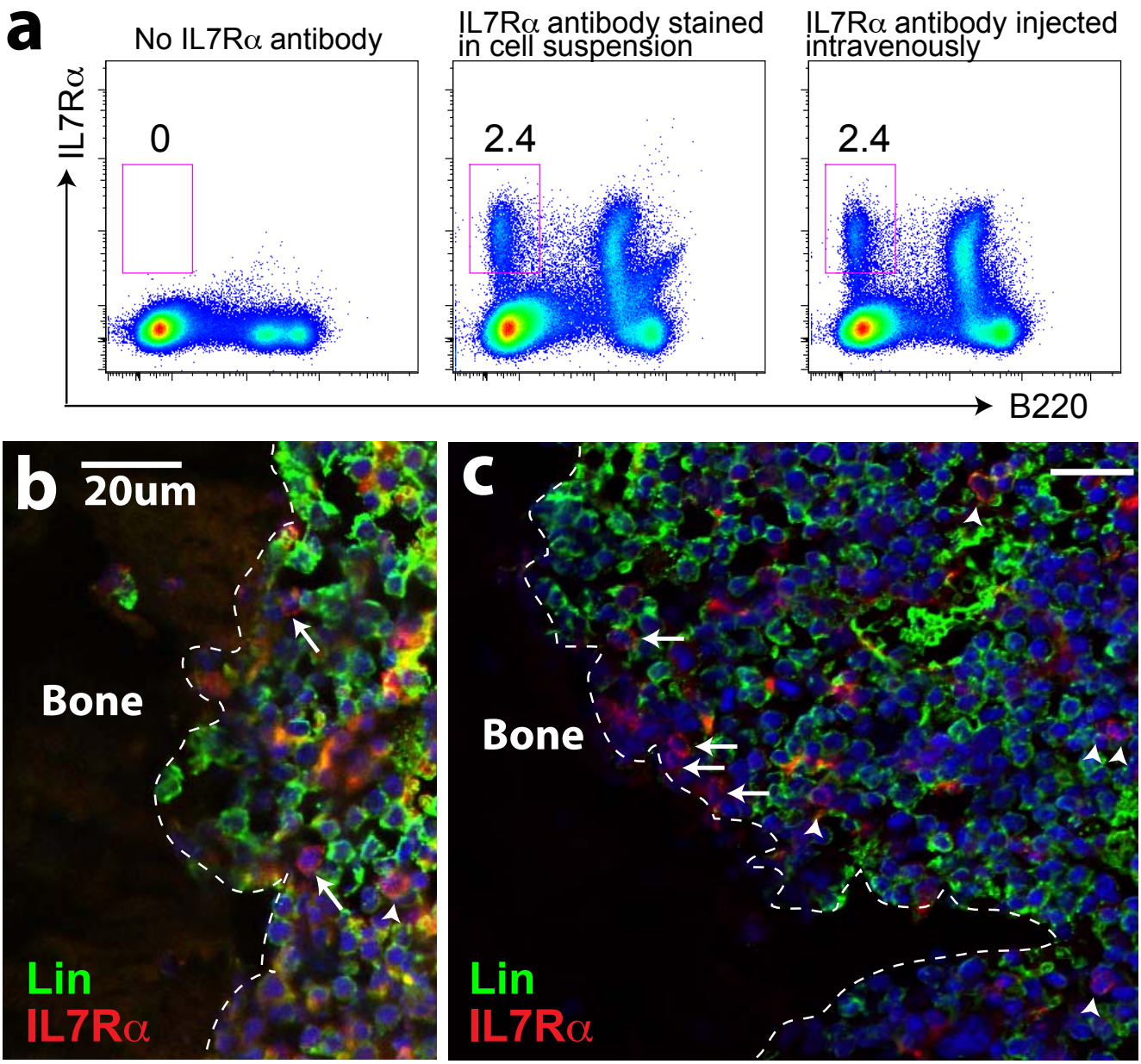
Supplementary Figure 6. *Cxcl12* from *Nestin-cre* expressing cells is not required for the maintenance of HSCs or restricted haematopoietic progenitors. a, *Nestin-cre; Cxcl12*$^{fl/fl}$ mice had normal bone marrow and spleen cellularity (n=4). **b**, *Nestin-cre; Cxcl12*$^{fl/fl}$ mice had normal bone marrow and spleen HSC frequencies (n=4). **c**, Bone marrow cells from *Nestin-cre; Cxcl12*$^{fl/fl}$ mice had normal reconstituting activity upon transplantation into irradiated mice (three experiments with a total of 14-15 recipients per genotype). **d-f**, *Nestin-cre; Cxcl12*$^{fl/fl}$ mice had normal frequencies of MPPs, LMPPs, CMPs, MEPs, GMPs (**d**), CLPs (**e**), and committed B lineage progenitors in the bone marrow (**f**) (n=4). **g-i**, *Nestin-cre; Cxcl12*$^{fl/fl}$ mice had normal frequencies of myeloerythroid colony-forming progenitors in the bone marrow (**g**), spleen (**h**), and blood (**i**) (n=3). Δ, recombined *Cxcl12*^{fl} allele; con, negative control mice with the following *Cxcl12* genotypes: +/+ or fl/+ or fl/fl without *cre*. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance.



Supplementary Figure 7. *Tie2-cre; Cxcl12$^{fl/fl}$* mice and *Lepr-cre; Cxcl12$^{fl/-}$* mice exhibited grossly normal haematopoiesis. a, White blood cell, red blood cell, and platelet counts in the blood of adult *Tie2-cre; Cxcl12$^{fl/fl}$* mice relative to littermate controls (n=5). **b**, Frequencies of B cells, T cells, myeloid cells, megakaryocyte lineage cells, and erythroid lineage cells in the bone marrow of adult *Tie2-cre; Cxcl12$^{fl/fl}$* mice relative to littermate controls (n=6-10). **c** and **d**, HSCs from *Tie2-cre; Cxcl12$^{fl/fl}$* mice had normal frequencies of BrdU^{+} cells (**c**, n=3-4) and Annexin V^{+} cells (**d**, n=3-4). **e**, Blood cell counts in adult *Lepr-cre; Cxcl12$^{fl/-}$* mice relative to littermate controls (n=4-7). **f**, Frequencies of B cells, T cells, myeloid cells, megakaryocyte lineage cells, and erythroid lineage cells in the bone marrow of adult *Lepr-cre; Cxcl12$^{fl/-}$* mice relative to littermate controls (n=3-6). Δ, recombined *Cxcl12^{fl}* allele; -, germline deleted allele; con, negative control mice with the following *Cxcl12* genotypes: +/+ or fl/+ or fl/fl or fl/- without cre. Data represent mean±s.d.. Two-tailed student's t-tests assessed statistical significance.



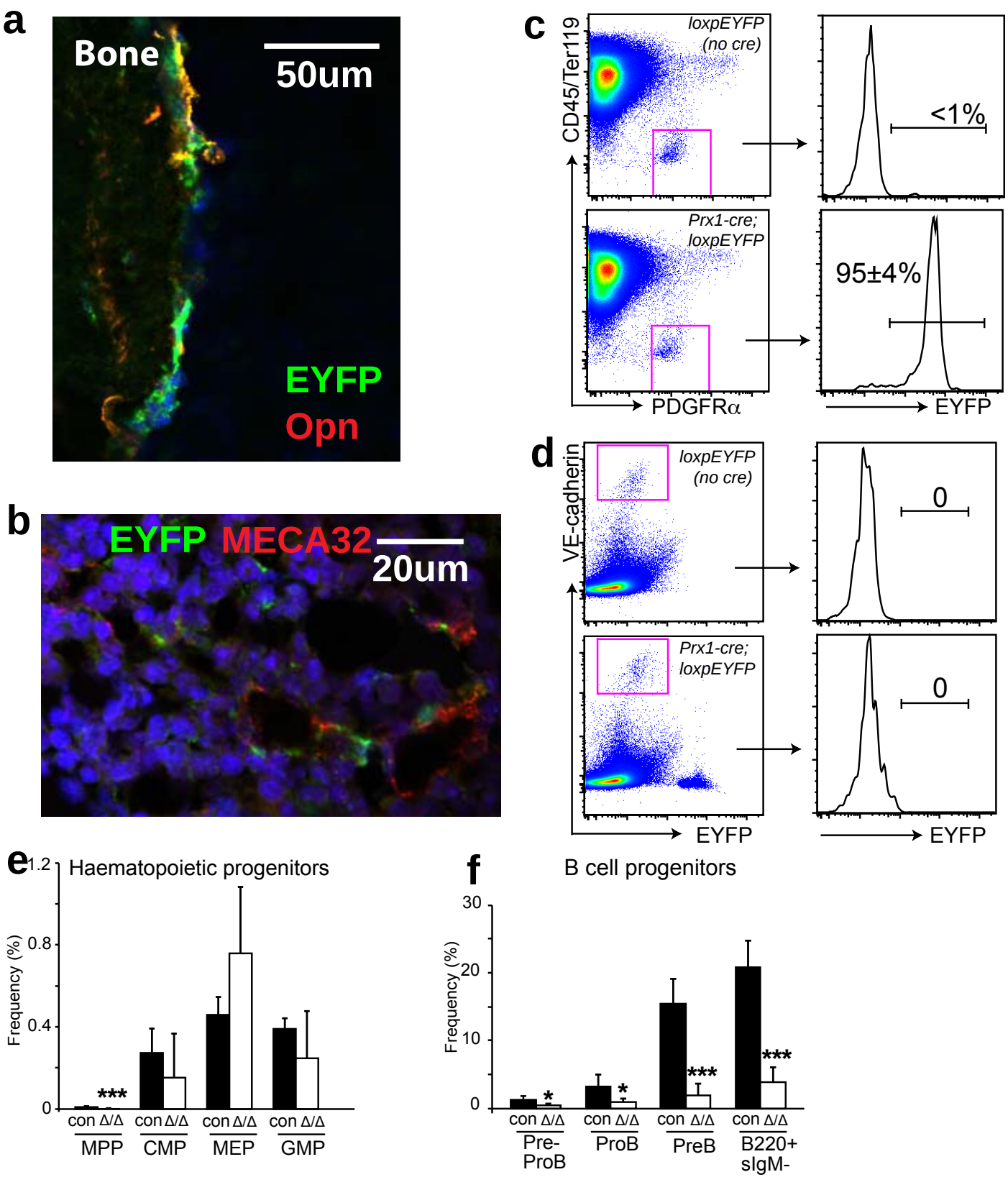
Supplementary Figure 8. *Col2.3-cre; Cxcl12$^{fl/fl}$* mice exhibited grossly normal haematopoiesis, and CLPs from adult *Col2.3-cre; Cxcl12$^{fl/fl}$* mice had a normal frequency of BrdU^{+} and Annexin V^{+} cells. a, Blood cell counts in adult *Col2.3-cre; Cxcl12$^{fl/fl}$* mice relative to littermate controls (n=5). **b**, Frequencies of B cells, T cells, myeloid cells, megakaryocyte lineage cells, and erythroid lineage cells in the bone marrow of adult *Col2.3-cre; Cxcl12$^{fl/fl}$* mice compared to littermate controls (n=4). **c**, In the competitive reconstitution experiments shown in Fig.4d, 20 HSCs from *Col2.3-cre; Cxcl12$^{fl/fl}$* mice did not exhibit significant differences in myeloid, B, or T lineage reconstituting capacity compared to littermate controls (three experiments with a total of 14-15 recipient mice per genotype).**d** and **e**, The thymus from *Col2.3-cre; Cxcl12$^{fl/fl}$* mice had normal cellularity (**d**) and normal frequency of early thymic progenitors (ETPs) (**e**, n=3). **f** and **g**, CLPs from adult *Col2.3-cre; Cxcl12$^{fl/fl}$* mice had normal frequencies of BrdU^{+} (**f**) and Annexin V^{+} cells (**g**, n=6). Δ, recombined *Cxcl12^{fl}* allele; -, germline deleted allele; con, negative control mice with the following *Cxcl12* genotypes: +/+ or fl/+ or fl/fl or fl/- without *cre*. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance.



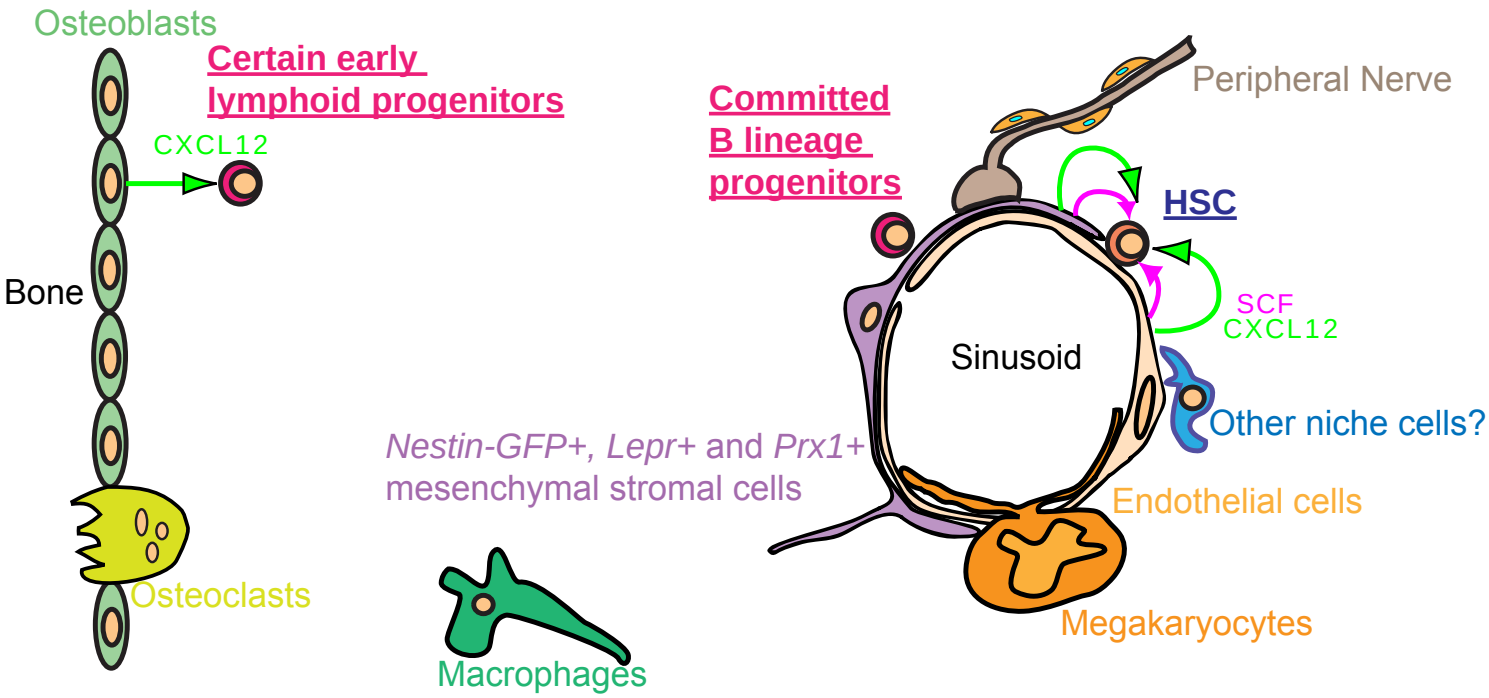
d

At the endosteal surface	Within 5 cell diameters of the endosteal surface	Other locations
30% (73/241)	13% (31/241)	57% (137/241)

Supplementary Figure 9. Localization of Lin$^-$IL7Rα$^+$ early lymphoid progenitors in bone marrow sections. a, Intravenous injection of a monoclonal antibody against IL7Rα rapidly stained IL7Rα$^+$ cells in the bone marrow. The staining pattern was the same as observed when bone marrow cells were stained in suspension, rather than by intravenous injection. **b** and **c**, Immunofluerescence images showing the in vivo localization of Lin$^-$IL7Rα$^+$ cells in bone marrow sections. Arrows point to cells that are adjacent to the endosteum or within 5 cell diameters of the endosteum. Arrowheads point to cells that are located in other regions of the bone marrow, away from the endosteum. **d**, Quantification of Lin$^-$IL7Rα$^+$ cell localization in bone marrow sections. Note that only 5% of all bone marrow cells were immediately adjacent to the endosteum in the sections we examined, such that Lin$^-$IL7Rα$^+$ cells were 6-fold more likely than the average bone marrow cell to be immediately adjacent to the endsteum (data not shown). Images from two independent experiments were quantified.



Supplementary Figure 10. *Prx1-cre; Cxcl12$^{fl/fl}$* mice exhibited a depletion of lymphoid progenitors. a, *Prx1-cre* recombined a *loxpEYFP* reporter in bone-lining osteoblasts. Osteoblasts were stained with an anti-osteopontin antibody. **b**, *Prx1-cre* recombined the *loxpEYFP* reporter in perivascular stromal cells that localized adjacent to MECA32^{+} endothelial cells. **c** and **d**, *Prx1-cre* recombined the *loxpEYFP* reporter in 95±4% of CD45/Ter119^{-}PDGFRα^{+} perivascular stromal cells (**c**) (n=3) but not in VE-cadherin^{+} bone marrow endothelial cells (**d**). **e**, *Prx1-cre; Cxcl12$^{fl/fl}$* mice had normal frequencies of CMPs, MEPs, and GMPs but a significantly reduced frequency of MPPs (n=3-4). **f**, *Prx1-cre; Cxcl12$^{fl/fl}$* mice exhbited a significant reduction in the frequency of committed B lineage progenitors in their bone marrow (n=4-5). Δ, recombined *Cxcl12^{fl}* allele; con, negative control mice with the following *Cxcl12* genotypes: +/+ or fl/+ or fl/fl without *cre*. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance. *P<0.05, **P<0.01, ***P<0.001.



Supplementary Figure 11. HSCs reside in a perivascular niche while certain early lymphoid progenitors reside in an endosteal niche. HSCs are found mainly adjacent to sinusoids in the bone marrow[4,9], where endothelial cells and perivascular stromal cells promote HSC maintenance by producing both SCF and CXCL12[10]. The perivascular stromal cells can be identified based on their expression of *Lepr*-Cre[10], *Prx1*-Cre[27], or *Nestin*-GFP transgene[12], but we have not been able to detect expression of endogenous *Nestin*, or *Nestin*-Cre or *Nestin*-CreER transgenes in these cells[10]. These perivascular stromal cells also strongly overlap with reticular cells that express high levels of Cxcl12[11] and CD45/Ter119$^-$PDGFRα$^+$ stromal cells that have been characterized as mesenchymal stem/progenitor cells[37]. It is likely that other cells also contribute to this niche, potentially including innervation by sympathetic nerves[38,39] and non-myelinating Schwann cells[40]. Osteoblasts do not express *Scf*[10] and express *Cxcl12* at levels 1000-fold lower than perivascular stromal cells and 10-fold lower than endothelial cells (Fig. 1p). Conditional deletion of *Scf* or *Cxcl12* from osteoblasts does not affect HSC frequency or function; however, osteoblasts are a physiologically important source of CXCL12 for the maintenance of some common lymphoid progenitors and IL7Rα$^+$LMPPs, demonstrating that certain early lymphoid progenitors reside in an endosteal niche that is spatially and cellularly distinct from HSCs. Data in our study and in prior studies[41] are consistent with the possibility that committed B lineage progenitors return to a perivascular niche.