# Connective tissue growth factor is expressed in bone marrow stromal cells and promotes interleukin-7-dependent B lymphopoiesis

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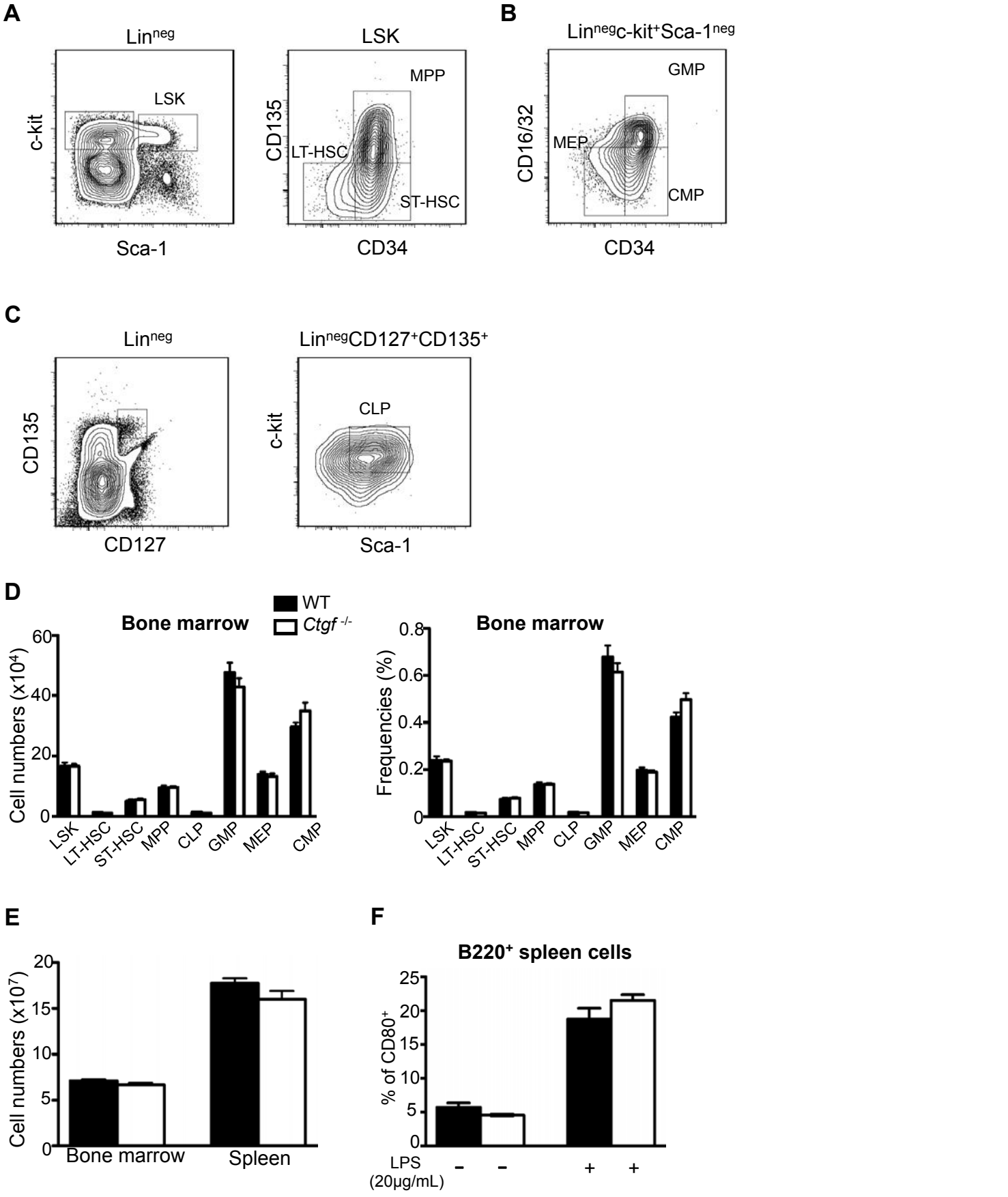
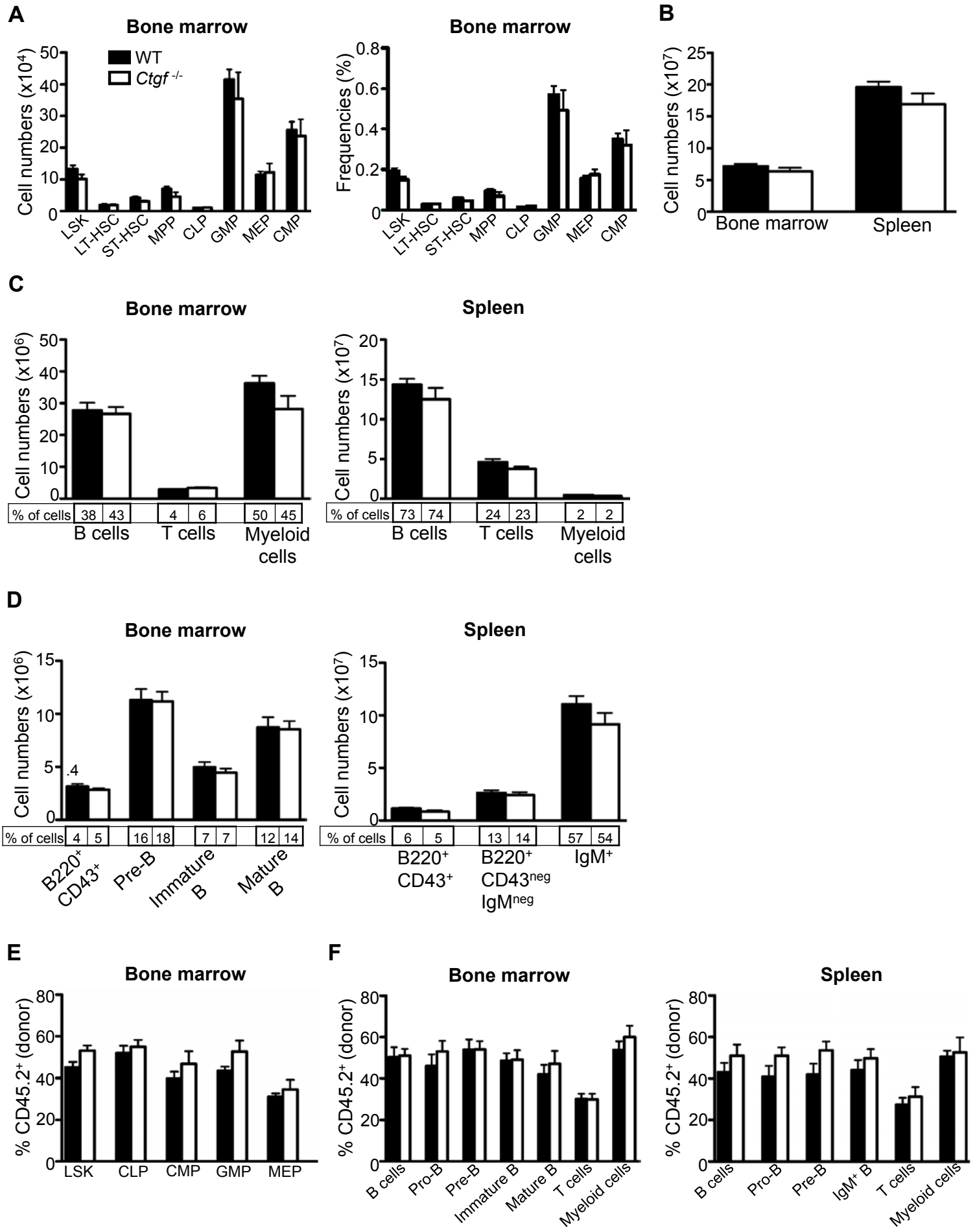
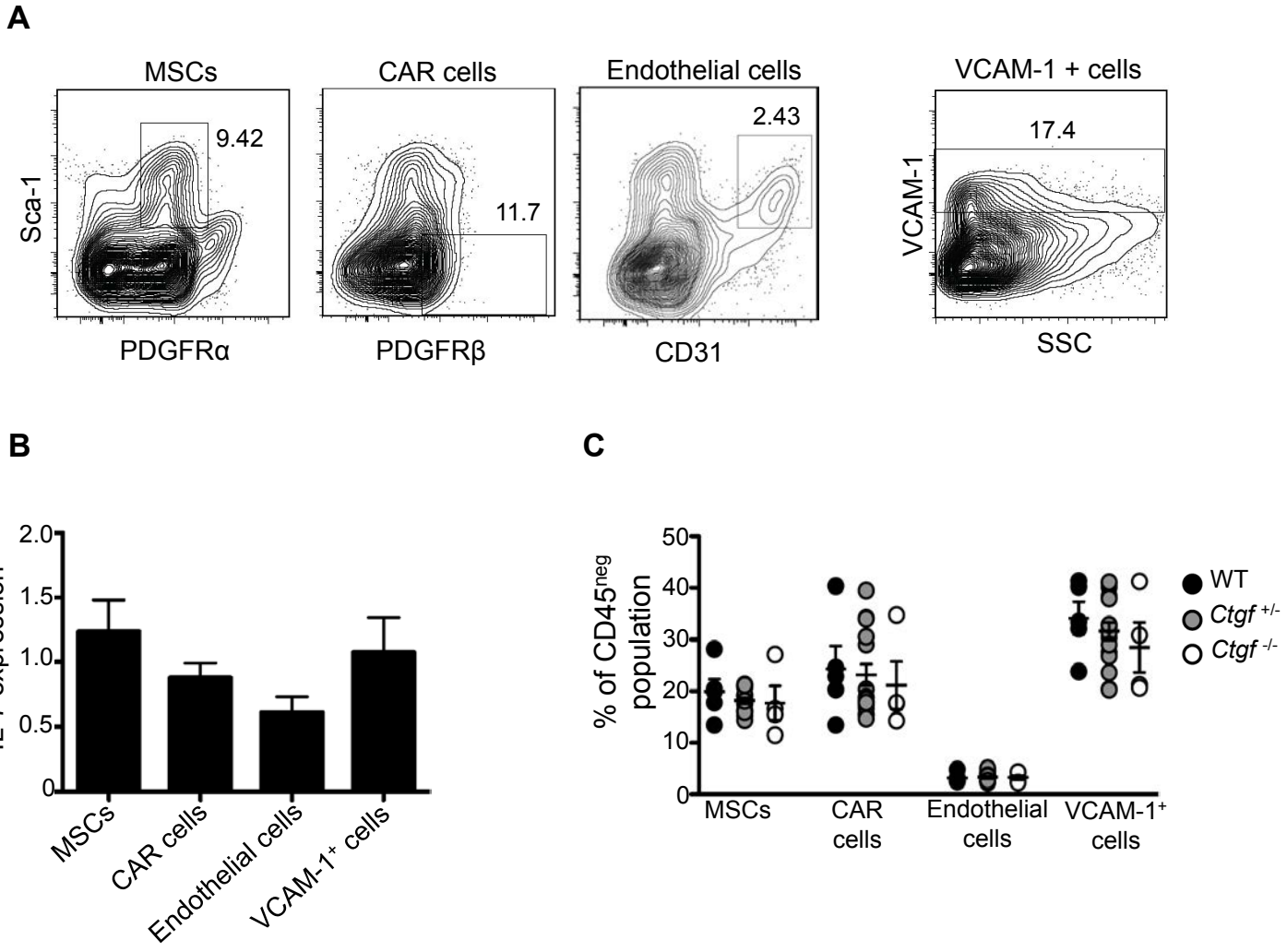
## Supplementary Figure S1



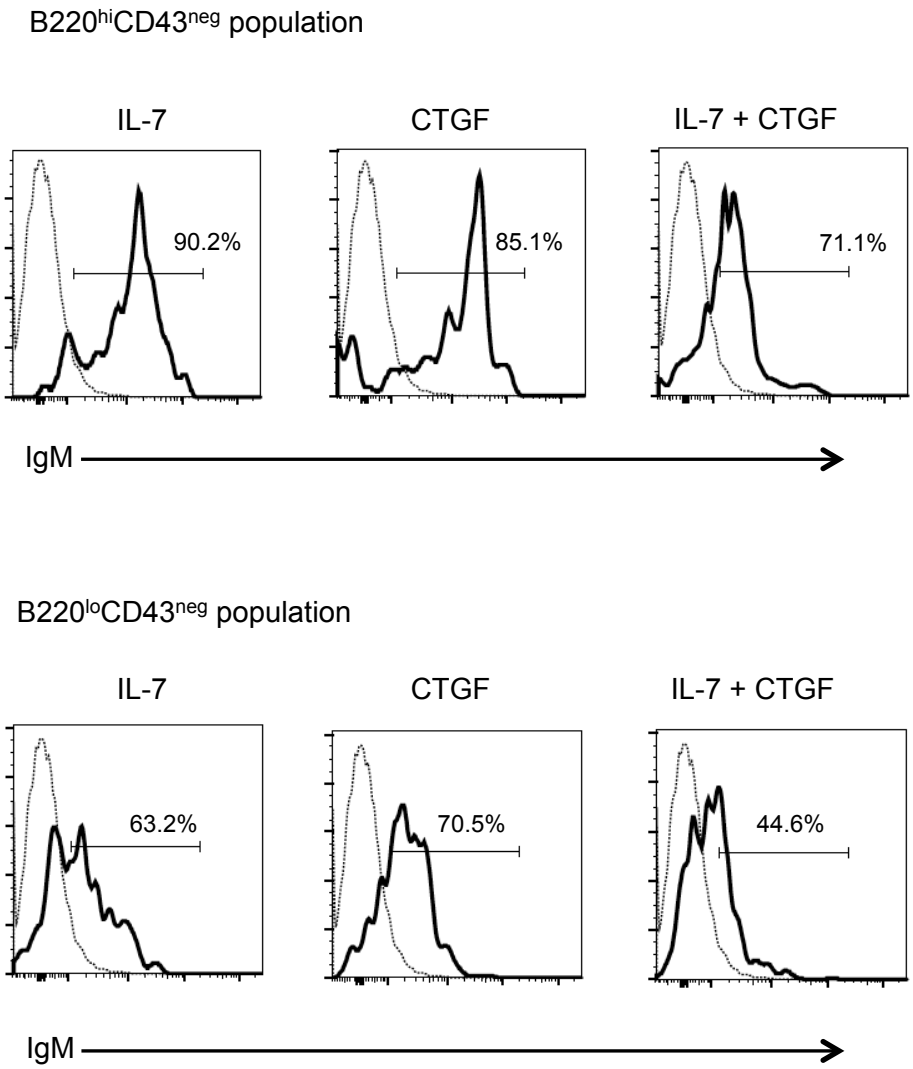
## Supplementary Figure S2



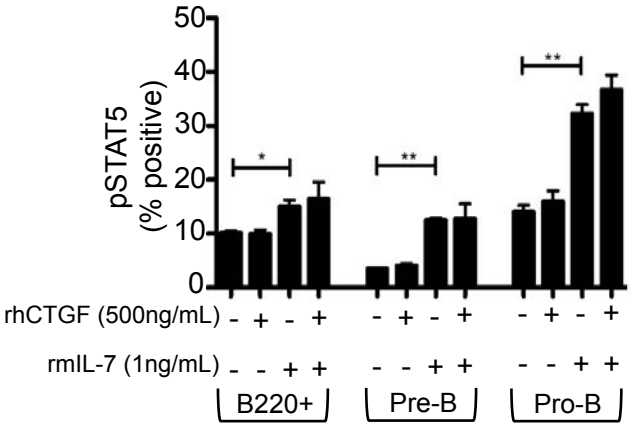
**Supplementary Figure S3**



**Supplementary Figure S4**



## Supplementary Figure S5



**Supplementary Figure Legends**

**Figure S1. The hematopoiesis of recipient mice transplanted with fetal liver cells.** $1x10^6$ total E13.5 fetal liver cells from WT (■) or *Ctgf* $^{-/-}$ (□) mice were transplanted into lethally irradiated WT recipient mice (n≥6 mice per group). (A) Gating strategy of hematopoietic stem and progenitor cells (HSPCs) in the BM: LSK (lineage (lin)$^{neg}$Sca-1$^+$c-kit$^+$), short-term HSC (ST-HSC) (LSK$^+$CD135$^{neg}$CD34$^+$), long-term HSC (LT-HSC) (LSK$^+$CD135$^{neg}$CD34$^{neg}$), and multipotent progenitor (MPP) (LSK$^+$CD135$^+$CD34$^+$). (B) Gating strategy of myeloid progenitors: granulocyte/monocyte progenitor (GMP) (lin$^{neg}$c-kit$^+$Sca-1$^{neg}$CD16/32$^+$CD34$^+$), megakaryocyte/erythroid progenitor (MEP) (lin$^{neg}$c-kit$^+$Sca-1$^{neg}$CD16/32$^{neg}$CD34$^{neg}$), and common myeloid progenitor (CMP) (lin$^{neg}$c-kit$^+$Sca-1$^{neg}$CD16/32$^{neg}$CD34$^+$). (C) Gating strategy of lymphoid progenitor: common lymphoid progenitor (CLP) (lin$^{neg}$CD127$^+$CD135$^+$c-kit$^{int}$Sca-1$^{int}$). Lineage markers in A-C were CD2, CD3ε, CD4, CD5, CD8a, CD19, B220, Gr-1, and Ter119. (D) Total number and frequency of HSPC from BM. (E) Total cell numbers in the BM and spleen. (F) B220$^+$ cells isolated from spleen were cultured with 20μg/mL LPS for 48 hours. B-cell activation was determined as percentage of CD80$^+$ cells following culture.

**Figure S2. *Ctgf* $^{-/-}$ HSCs show no cell-autonomous defect and competitive disadvantage**. (A-D) $1x10^4$ LSK cells sorted from E13.5 WT (■) or *Ctgf* $^{-/-}$ (□) fetal liver were transplanted into lethally irradiated recipient WT mice. Data shown illustrate mean +/- SEM cellular composition of BM and spleen at 16 weeks post transplantation. Data from two independent experiments with n=4

mice per group. (A) Total number and frequency of HSPC from BM as per Supplementary Figure S1. (B) Total cell numbers. (C) Total cell numbers and frequencies in BM and spleen of B cells, T cells, and myeloid cells. (D) Total number and frequency of $B220^{+}CD43^{+}$ (pro-B), pre-B, immature B, and mature B cells in BM as well as $B220^{+}CD43^{+}$, $B220^{+}CD43^{neg}IgM^{neg}$, and $IgM^{+}$ B ($B220^{+}CD43^{neg}IgM^{+}$) populations in spleen. (E-F) Equal numbers (5000) of $CD45.2^{+}$ donor cells (WT(■) or *Ctgf*$^{-/-}$ (□)) and competitor cells ($CD45.1^{+}$) were transplanted into the recipients (n=5-8 mice per group). Percentage of reconstituted $CD45.2^{+}$ donor cells was measured at 16 weeks post transplantation. Error bars represent SEM. (E) HSPC compartment. (F) BM and spleen.

**Figure S3. The bone marrow stromal cells of newborn mice.** (A) Flow cytometric profiles of different types of BMSC: MSCs ($Sca\text{-}1^{+}PDGFR\alpha^{+}$), CAR cells ($Sca\text{-}1^{neg}PDGFR\beta^{+}$), endothelial cells ($Sca\text{-}1^{+}CD31^{+}$), and VCAM-$1^{+}$ reticular cells. (B) IL-7 expression levels, normalized to expression of *Eef1a1*, were determined by qRT-PCR in newborn BM cells from femurs and tibias. (C) Composition of BMSCs from WT (●), *Ctgf*$^{+/-}$ (◐) and *Ctgf*$^{-/-}$ (○) newborn mice, data illustrate individual newborn mice collected from 3 separate pregnancies.

**Figure S4. Analysis of the sIgM expression of B cells after 48 hours culture.** Representative histogram of the sIgM level in $B220^{hi}CD43^{neg}$ and $B220^{lo}CD43^{neg}$ cells after incubating pro-B cells with rhCTGF, rmIL-7, or both for 48 hours. Dotted line, isotype control; solid line, anti-mouse IgM antibody.

**Figure S5. CTGF regulation of B-cell development is independent of IL-7R/STAT5 signaling.** BM cells were incubated with rhCTGF, rmIL-7, or both for 30 minutes. The level of pSTAT5 was measured by flow cytometry. Pro-B and pre-B cells were defined as $B220^{+}CD43^{+}$ and $B220^{lo}CD43^{neg}$, respectively. Data were collected from two independent experiments with n=4 mice per group. Error bars represent SEM *, $p<0.01$; **, $p<0.001$

## Supplementary Table S1

Fluorochrome- or biotin-conjugated anti-mouse antibodies used in this study

| Study | Antibody | Clone |
|---|---|---|
| **Phenotypic analysis of mouse bone marrow, liver, and spleen** | PE-conjugated B220 | RA3-6B2 |
| | FITC-conjugated CD43 | S7 |
| | PE-Cy7-conjugated IgM | R6-602 |
| | PE-conjugated CD11b | M1/70 |
| | FITC-conjugated Gr-1 | RB6-8C5 |
| | FITC-conjugated CD3ε | 145-2C11 |
| **Isolation of B-cell subpopulations** | APC-conjugated B220 | RA3-6B2 |
| | PE-conjugated CD43 | S7 |
| | PerCP-Cy5.5-conjugated IgM | R6-602 |
| **Biotinylated antibodies for exclusion of lineage positive cells** | | |
| For fetal liver transplantation | Gr-1 | RB6-8C5 |
| | Ter119 | |
| For hematopoietic and progenitor cells (HSPC) analysis | B220 | RA3-6B2 |
| | CD2 | RM2-5 |
| | CD3ε | 145-2C11 |
| | CD4 | GK1.5 |
| | CD5 | 53-7.3 |
| | CD8a | 53-6.7 |
| | CD19 | 1D3 |
| | Gr-1 | RB6-8C5 |
| | Ter119 | |
| For bone marrow stromal cell (BMSC) studies | CD45 | 30-F11 |
| | CD11b | M1/70 |
| | Ter119 | |
| **Analysis subtypes of HSPC** | PE-Cy7-conjugated Sca-1 | D7 |
| | FITC-conjugated CD34 | RAM34 |
| | PE-conjugated CD135 | A2F10.1 |
| | APC-Cy7-conjugated c-kit | 2B8 |
| | FITC-conjugated CD127 | DB/199 |
| | PE-conjugated CD16/32 | 2.4G2 |
| **Analysis subtypes of BMSC** | APC-Cy7-conjugated Sca-1 | D7 |
| | FITC-conjugated CD31 | MEC13.3 |
| | APC-conjugated PDGFRα | APA5 |
| | PE-conjugated PDGFRβ | APB5 |
| | PE-Cy7-conjugated VCAM-1 | MVCAM.A |

## Supplementary Methods

### Mice

Genotyping for *Ctgf* was performed by using PCR with primers 5'TGTGTAGGACTTCATTCAGTTCT3' and 5'GTCTGTGATCGCAGCTCACTC3' for *Ctgf* and 5'TGTGTAGGACTT-CATTCAGTTCT3' and 5'ATGGCCGCTTTTCTGGATTC3' for the neomycin insert with cycling conditions 94°C for 2.5 minutes, followed by 40 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 60 seconds, and final 72°C extension for 2 minutes.

### Photomicrograph

The images were taken by Leica DM2500 microscope with Leica DFC290HD camera and Leica Fluotar lenses in 5x magnification and 0.75 aperture at 25°C. The acquisition software was Leica Application Suite v4.1 and the contrast and brightness of images were adjusted by GIMP 2.6.10.

### Flow cytometry and cell sorting

For phenotypic analysis of mouse bone BM, liver, spleen, and isolation of B-cell subpopulations, combinations of mouse-specific antibodies were used (Supplementary Table S1). Fc block (2.4G2) was used to inhibit nonspecific binding. In transplantation studies we used PerCP-Cy5.5-conjugated CD45.2 (104) and APC-conjugated CD45.1 (A20; eBioscience) to identify the allelic markers. In the HSPC analysis, exclusion of lineage (lin)-positive cells was performed using a cocktail of biotinylated antibodies (Supplementary Table S1), followed by streptavidin-APC. Subtypes of hematopoietic stem and progenitor

cell were identified using combinations of antibodies (Supplementary Table S1). For the analysis of BMSCs, femurs and tibias were crushed and incubated with 337.5U/mL collagenase (Worthington) and 40U/mL DNaseI (Sigma-Aldrich) at 37°C for 45 minutes. BMSCs were purified by exclusion of hematopoietic cells using biotinylated antibodies (Supplementary Table S1) followed by streptavidin-PE-Cy5. Antibodies to identify subtypes of BMSC are listed in supplementary Table S1.

**Phosphorylated STAT5 intracellular flow cytometry**

BM cells were extracted from adult C57BL/6J mice, washed, and resuspended in αMEM. Cells were incubated for 30 minutes at 37°C in the presence of 500ng/mL recombinant human CTGF and/or 1ng/mL recombinant mouse IL-7 (eBioscience). Cells were then fixed and permeabilized with BD Phosflow Lyse/Fix buffer and Perm Buffer III according to manufacture's instructions (BD Bioscience). Cells were stained with APC-conjugated B220 (RA3-6B2), FITC-conjugated CD43 (S7), and PE-conjugated Stat5 (pY694) for 60 minutes before flow cytometric analysis.

**Fetal liver transplantation**

7- to 12-week old B6.SJL-Ptprca Pep3b/BoyJ (C57BL/6J-CD45.1) mice were used as recipients for all transplantation experiments. They were given acidified water with 1.1g/L neomycin sulfate and $1x10^6$U/L polymyxin B sulfate from 5 days before to 4 weeks after transplantation. They were irradiated with two doses of 550cGy with a two-hour interval between doses prior to transplantation.

**B-cell functional assay**

$B220^+$ spleen cells were isolated using B220 microbeads on the autoMACS separator (Miltenyi Biotec) achieving purity >94%. $1x10^6$ cells/ well were cultured in 24-well plates in the presence of 20μg/mL lipopolysaccharide (LPS) (Sigma-Aldrich) in αMEM (Life Technologies) supplemented with 10% fetal calf serum. After 48 hours, cells were stained with APC-conjugated B220 (RA3-6B2) and biotin-conjugated CD80 (16-10A1), followed by labeling with streptavidin-PE-Cy5 for flow cytometric analysis.

**B-cell maturation assay**

To measure maturation, pro-B ($B220^+CD43^+IgM^{neg}$) cells were isolated from adult C57BL/6J BM by FACS. $3x10^4$/well pro-B cells were cultured in 96-well plates in the presence of 500ng/mL rhCTGF and/or 1ng/mL rmIL-7 in αMEM supplemented with 10% fetal calf serum for 48 hours. Cells were harvested and subpopulations of B cell were measured using antibodies listed in supplementary Table S1.