# B-lymphopoiesis is stopped by mobilizing doses of G-CSF and is rescued by overexpression of the anti-apoptotic protein Bcl2

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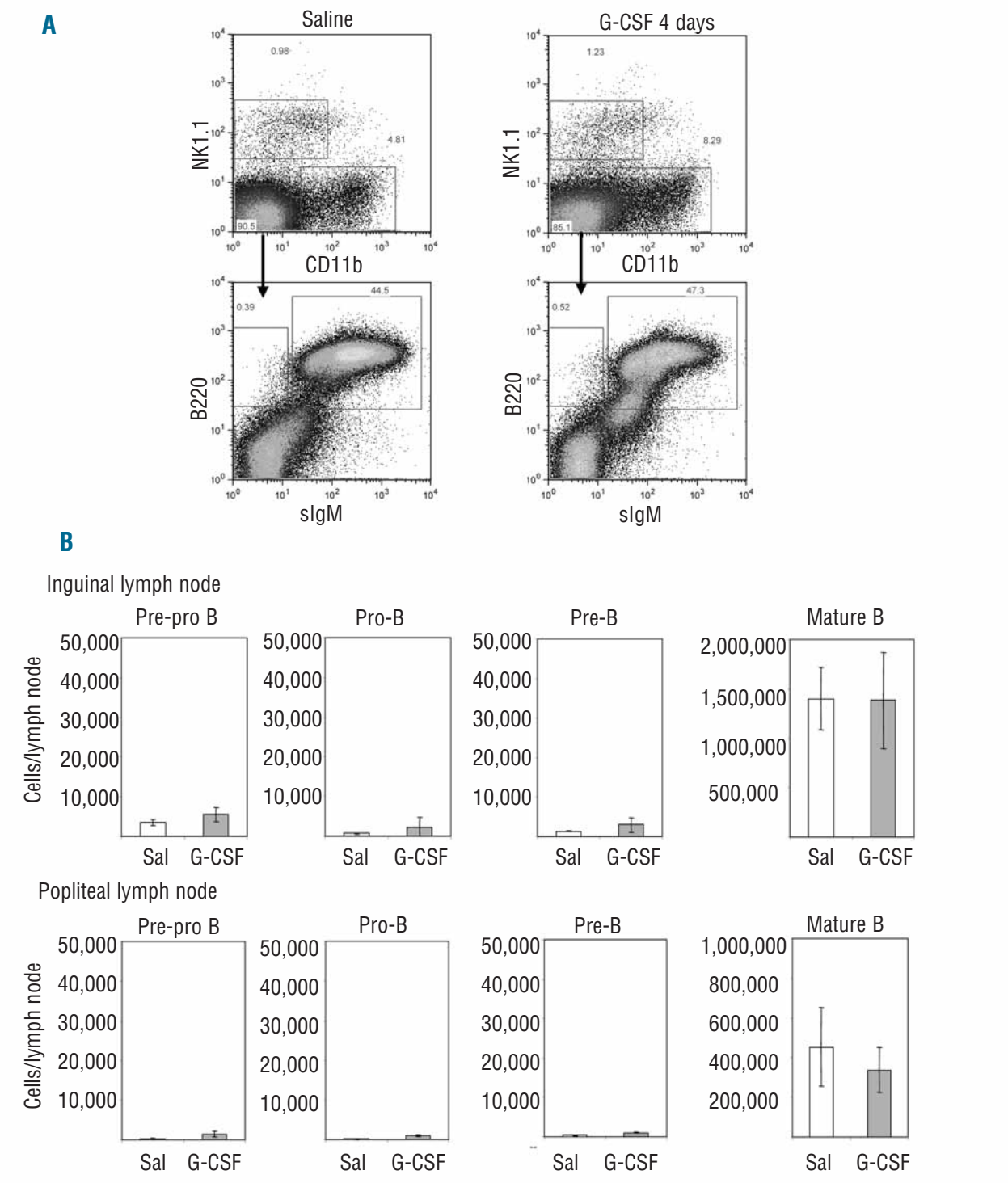
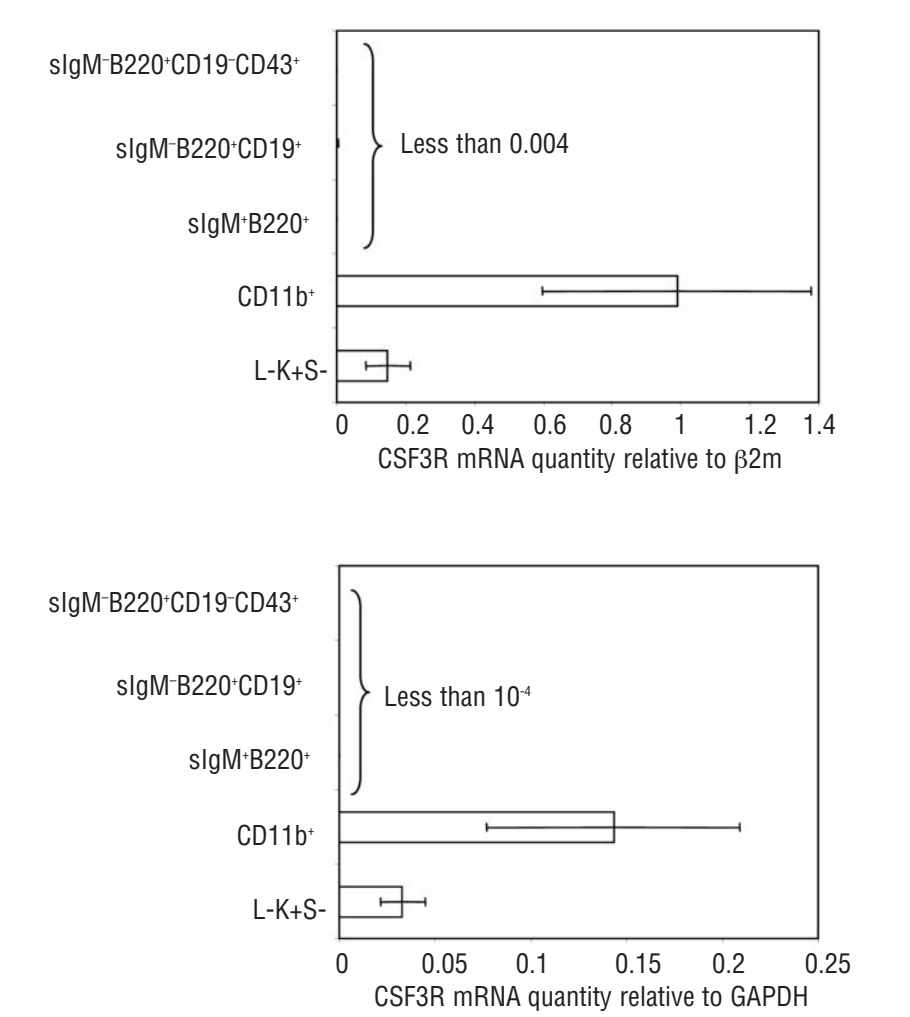
**Online Supplementary Table 1.** Primers and probes used in RT-qPCR analyses.

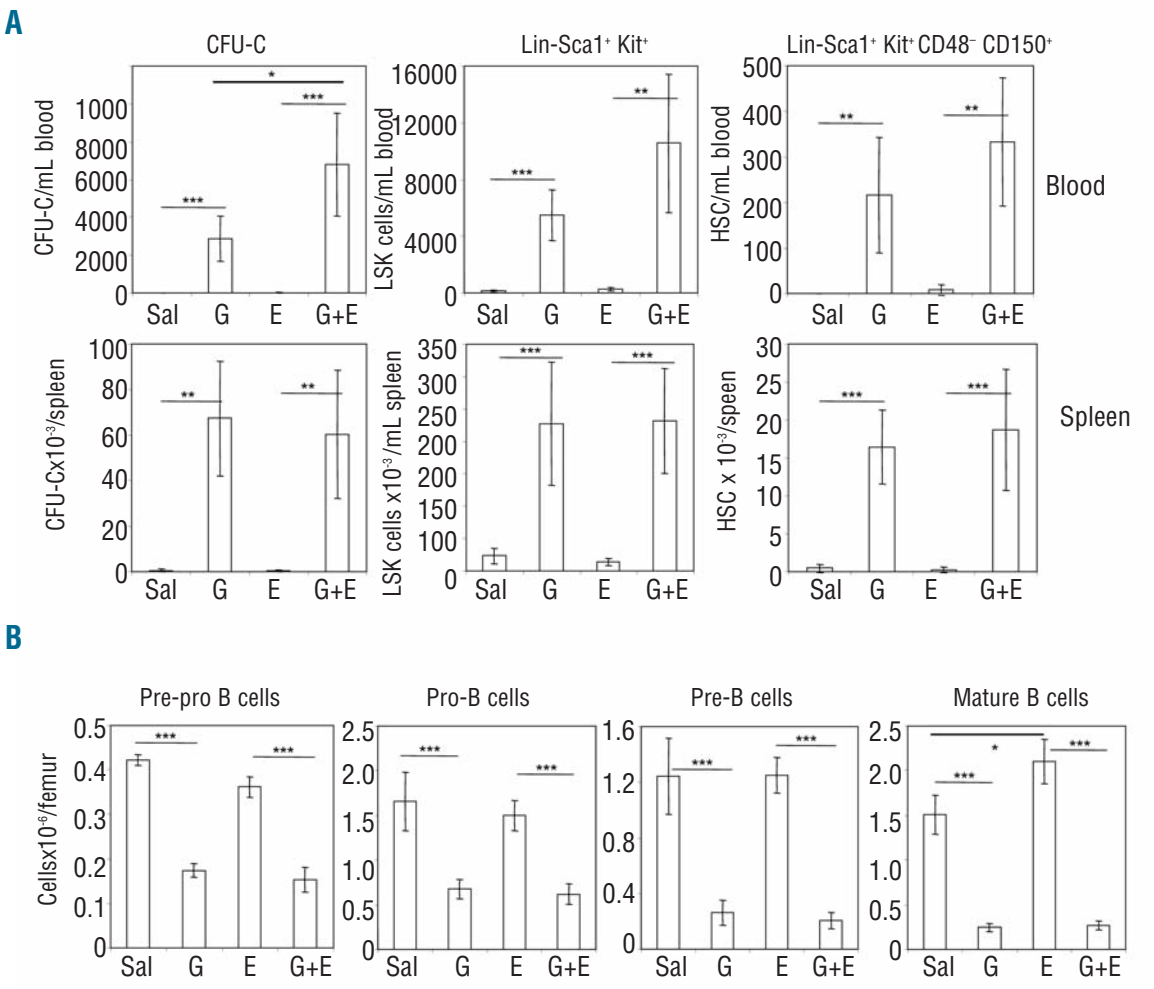
| | |
|---|---|
| IL-7 | forward (218f) 5'-TCATCATGACTACACCCACCTC-3'<br>reverse (313b) 5'-ACAGGCAGCAGAACAAGGAT-3'<br>probe (240f) FAM-5'-CCGCAGACCATGTTCCATGTTTCT-3'-BHQ1. |
| CXCL12 | forward (198f) 5'-GAGCCAACGTCAAGCATCTG-3'<br>reverse (298b) 5'-CGGGTCAATGCACACTTGTC-3'<br>probe FAM-5'- TCCAAACTGTGCCCTTCAGATTGTTGC-3'-BHQ |
| β2-microglobulin | Forward 5'-TTCACCCCCACTGAGACTGAT-3'<br>reverse 5'-GTCTTGGGCTCGGCCATA-3'<br>probe (94f) FAM-5'- CACTGACCGGCCTGTATGCTATCCA-3'-BHQ1 |
| GAPDH | Forward 5'-TGCACCACCAACTGCTTAGC-3'<br>reverse 5'-GGCATGGACTGTGGTCATGAG-3' |



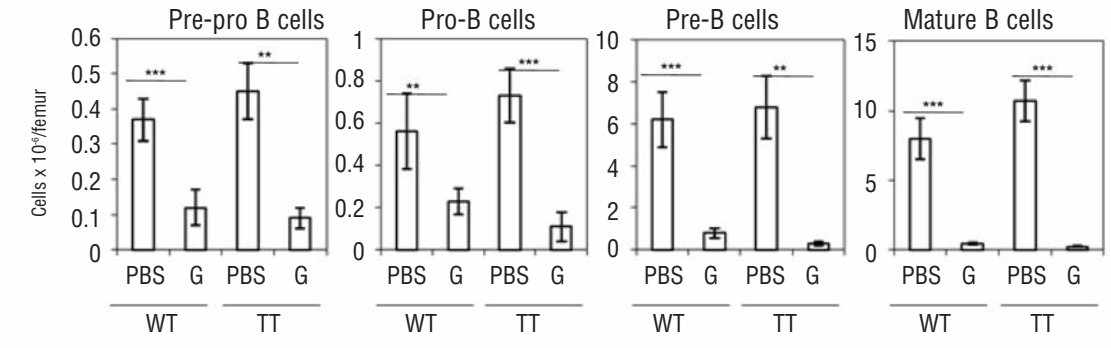

**Online Supplementary Figure S1.** G-CSF does not mobilize B cells to lymph nodes. C57BL/6 mice were injected with saline or G-CSF for 4 days. Inguinal and popliteal lymph nodes were harvested, dissociated and cells processed for flow cytometry analysis. (A). Flow cytometry profile of B cells in inguinal lymph nodes from mice injected for 4 days with saline or G-CSF. (B). Numbers of phenotypic sIgM$^-$ B220$^+$ CD19$^-$ CD43$^+$ pre-pro-B, sIgM$^-$ B220$^{low}$ CD19$^+$ CD43$^-$ pro-B, sIgM$^-$ B220$^+$ CD19$^+$ pre-B and mature sIgM$^+$ B220$^+$ B cells in inguinal and popliteal lymph nodes (mean±SD, 4 mice per group). There was no significant difference between saline and G-CSF treated group in any of the investigated populations.



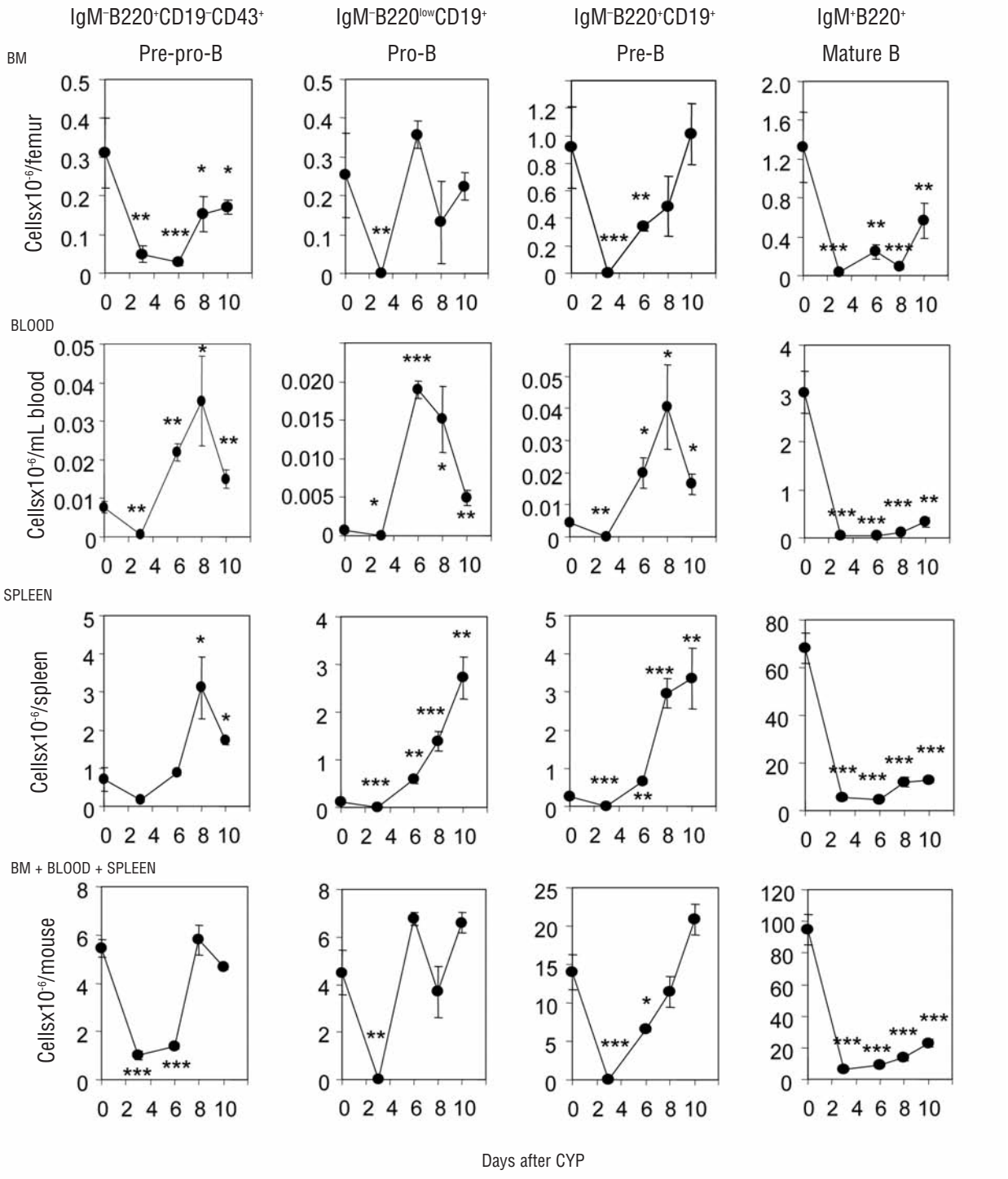

**Online Supplementary Figure S2.** G-CSF receptor mRNA is not transcribed by B-cell progenitors in the BM. Phenotypic sIgM⁻ B220⁺ CD19⁻ CD43⁺ pre-pro-B, sIgM⁻ B220⁺ CD19⁺ pro-B and pre-B cells, mature sIgM⁺ B220⁺ B cells were sorted from untreated mice. RNA was extracted and G-CSF receptor mRNA quantified by RT-qPCR relative to β2-microglobulin (top panel) or GAPDH (bottom panel). CD11b⁺ myeloid cells and Lin⁻Kit⁺Sca1⁻ myeloid progenitors were sorted as positive controls. Data are meam±SD from 4 separate sorts from 4 different mice.



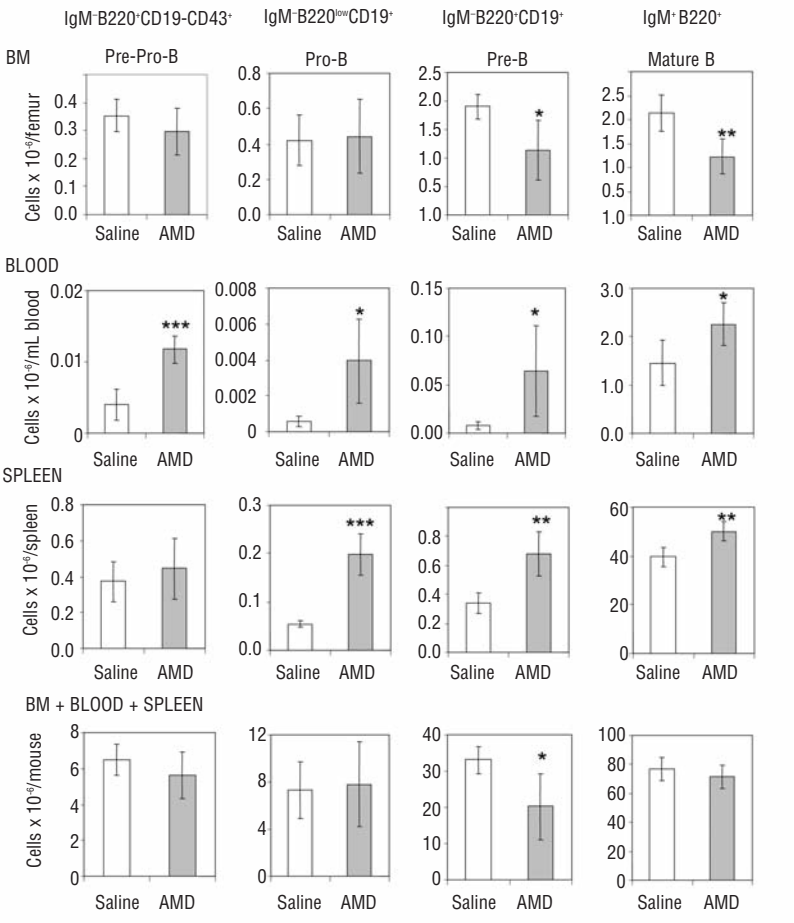

**Online Supplementary Figure S3.** Blockade of endogenous TNF-α with etanercept does not prevent mobilization of HSPC and IB lymphopoiesis arrest in response to G-CSF. Groups of 5 mice were treated with either saline, G-CSF alone (G), etanercept alone (E) or G-CSF together with etanercept (G+E) for 3 days. G- CSF treatment was for 3 days, etanercept for 4 days before tissue sampling. (A) Number of CFU-C, Lin- Sca1+ kit+ HSPC and Lin- Sca1+Kit+ CD48- CD150+ HSC were measured in blood and spleen. (B) Number of pre-pro-B, pro-B, pre-B and mature B cells were measured in the BM. Data are mean±SD. ***$P$<0.001, **$P$<0.01 and *$P$<0.05.




**Online Supplementary Figure S4.** Deletion of the *TNF-α* and *TRAIL* genes does not prevent effect of G-CSF on medullar B-lymphopoiesis. Cohorts of wild-type mice (WT) and mice double knock-out for TNF-α and TRAIL (TT) were treate for 4 consecutive days with G-CSF (G) or with PBS (PBS). BM were harvested the next morning and the number of pre-pro-B, pro-B, pre-B and mature B cells measured. ***$P$<0.001, **$P$<0.01 and *$P$<0.05.




**Online Supplementary Figure S5.** Medullar B lymphopoiesis is not stopped during CYP-induced mobilization. The number of cells in each B-cell subset (pre-pro-B, Pro-B, pre-B and mature B cells) was measured by flow cytometry in BM, blood and spleen at indicated time points after a single CYP injection at Day 0. Results per mouse were calculated after summation of content in BM, blood and spleen. The number of CFU-B was measured in B-cell colony assays. Data are average±SD of 4 mice per time point per treatment group. ***$P$<0.001, **$P$<0.01, and *$P$<0.05.




**Online Supplementary Figure S6.** Medullar B lymphopoiesis is not stopped during AMD3100-induced mobilization. The number of cells in each B-cell subset (pre-pro-B, Pro-B, pre-B and mature B cells) was measured by flow cytometry in BM, blood and spleen 1 h after AMD3100 administration. Results per mouse were calculated after summation of content in BM, blood and spleen. The number of CFU-B was measured in B-cell colony assays. Data are average±SD of 4 mice per time point per treatment group. ***$P$<0.001, **$P$<0.01, and *$P$<0.05.