Supporting Information

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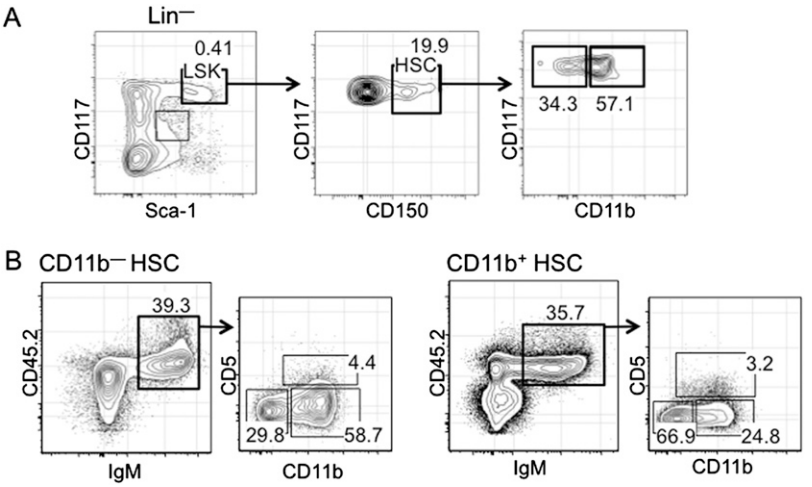

Fig. S1. CD11b expression does not segregate with B-1 progenitor potential in hematopoietic stem cells (HSCs). (*A*) Representative FACS strategy used to isolate CD11b$^+$ and CD11b$^-$ CD150$^+$ HSCs. The frequency of lineage negative (Lin$^-$) Sca-1$^+$ c-kit$^+$ (LSK) cells in total bone marrow is indicated in *Left*. The LSK cells were gated according to the phenotypes shown in *Center* and *Right*. (*B*) CD11b$^+$ and CD11b$^-$ HSCs from 15-wk-old adult mice were purified and injected in vivo into CD45.1 *Rag2*$^{-/-}$ mice that were preconditioned with 500 R from a 137^{Cs} irradiator (120 R/min, Mark I-68A; JL Shepherd and Associates) 24 h earlier. Production of donor CD45.2$^+$ sIgM^{high} CD11b$^+$ CD5$^+$ B-1a and sIgM^{high} CD11b$^+$ CD5$^-$ B-1b cells in the peritoneal cavity was evaluated 6 wk later. The frequency of cells within individual gates is indicated on the plots.

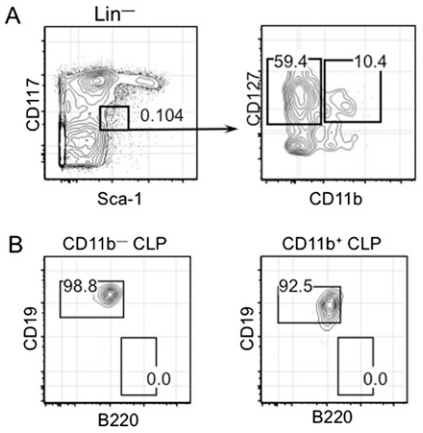

Fig. S2. CD11b expression does not segregate with B-1 progenitor potential in lymphoid progenitors (CLPs). (*A*) Representative FACS strategy used to isolate CD11b$^+$ and CD11b$^-$ CLPs. The frequency of LSK cells in total bone marrow is indicated in *Left*. The LSK cells were also gated according to the phenotype shown in *Right*. (*B*) CD11b$^+$ and CD11b$^-$ CLPs from 2.5-wk-old neonates generate B-1 progenitors in clonal cultures. Single CLPs were deposited into wells of 96-well microtiter plates by the automatic cell deposition unit on a FACSaria (Becton-Dickinson). Cells were cultured in RPMI 1640, 10% FCS, 5×10^{-5} M 2-β-mercaptoethanol, 1 mM L-glutamine, 100 U/mL streptomycin, 100 μg/mL penicillin, 50 μg/mL gentamycin, 20 ng/mL IL-3, 20 ng/mL IL-6, 20 ng/mL SCF, 20 ng/mL Flt-3 ligand, and 20 ng/mL IL-7 (Biosource International) for 4–6 d in a humidified incubator at 37 °C and 5% CO_2/air. Cells were then harvested and tested for their B-1 progenitor phenotype by immunostaining and analysis with the high-throughput sampler 96-well plate adapter for the BD LSR II flow cytometer. A total of 145 and 292 individual cells were tested for CD11b$^-$ and CD11b$^+$ CLP, respectively.

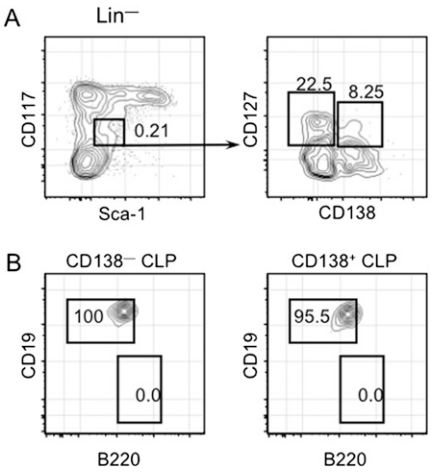

Fig. S3. CD138 expression does not segregate with B-1 progenitor potential in CLPs. (*A*) Representative FACS strategy used to isolate $CD138^+$ and $CD138^-$ CLPs. The frequency of LSK cells in total bone marrow is indicated in *Left*. The LSK cells were also gated according to the phenotype shown in *Right*. (*B*) $CD138^+$ and $CD138^-$ CLPs from 2.5-wk-old neonates generate B-1 progenitors in clonal cultures. Single CLPs were deposited into wells of 96-well microtiter plates by the automatic cell deposition unit on a FACSaria (Becton-Dickinson). Cells were cultured in RPMI 1640, 10% FCS, 5×10^{-5} M 2-β-mercaptoethanol, 1 mM L-glutamine, 100 U/mL streptomycin, 100 μg/mL penicillin, 50 μg/mL gentamycin, 20 ng/mL IL-3, 20 ng/mL IL-6, 20 ng/mL SCF, 20 ng/mL Flt-3 ligand, and 20 ng/mL IL-7 (Biosource International) for 4–6 d in a humidified incubator at 37 °C and 5% CO_2/air. Cells were then harvested and tested for their B-1 progenitor phenotype by immunostaining and analysis with the high-throughput sampler 96-well plate adapter for the BD LSR II flow cytometer. A total of 96 individual cells were tested for both $CD138^-$ and $CD138^+$ CLP, respectively.

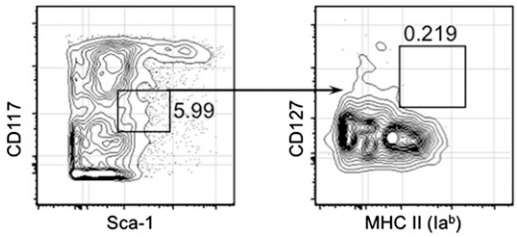

Fig. S4. CLPs do not express MHC class II antigens. Representative FACS strategy to identify MHC II expression on CLPs. Fresh, whole bone marrow from 4-wk-old B6 mice was stained and analyzed on the BD LSRII flow cytometer. The frequency of LSK cells in total bone marrow is indicated in *Left*. The LSK cells were also gated according to the phenotype shown in *Right*.