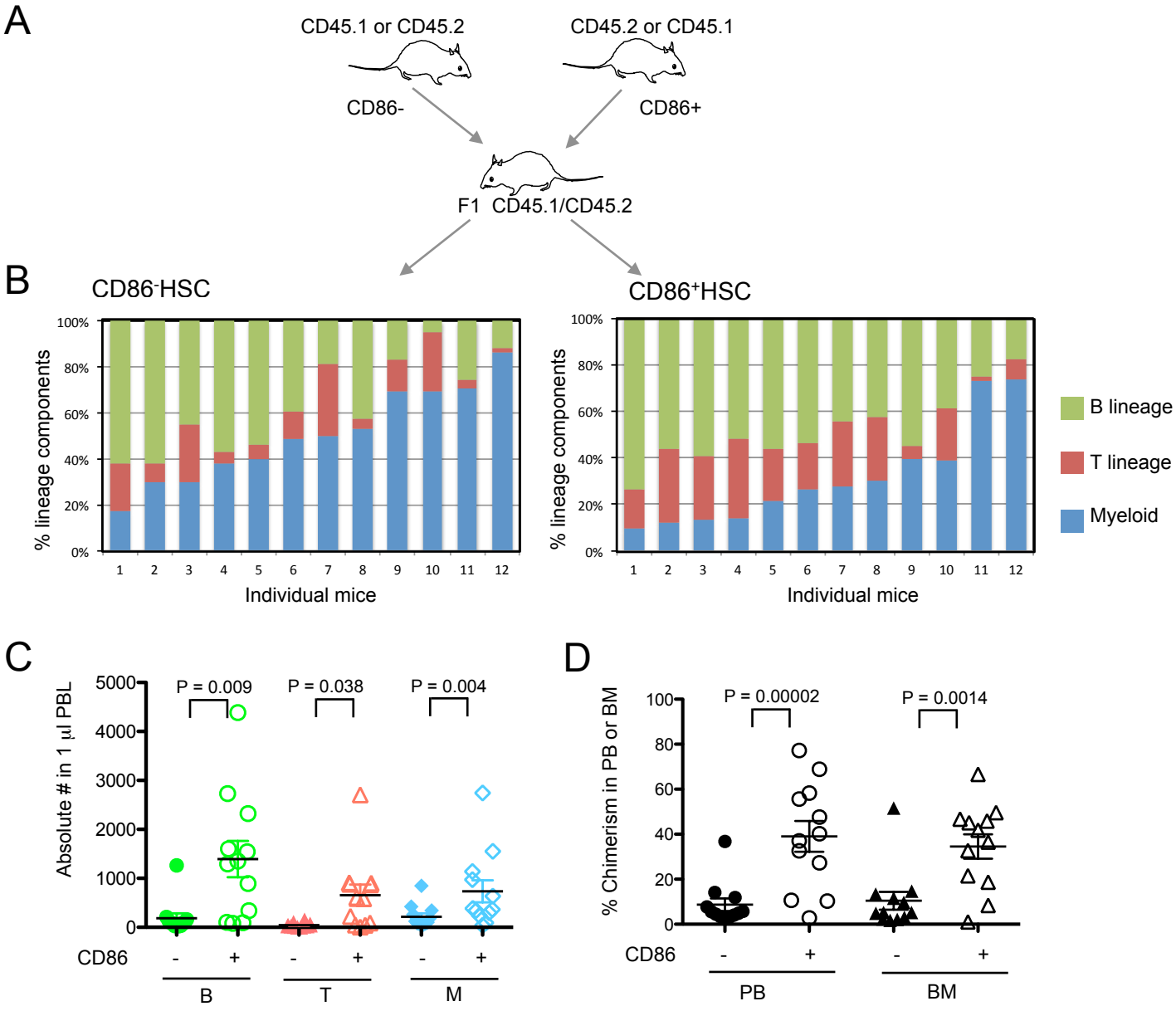

Figure S1. CD86^{-} CD150^{Hi} HSCs are poor at restoring the adaptive immune system. **(A)** A schematic representation of the experimental design is shown. Two hundred CD86^{-} and CD86^{+} CD150^{Hi} HSCs from 10 mo old mice were transplanted together into CD45.1/ CD45.2 F1 mice along with 2 x 10^5 whole bone marrow cells. The donor type cells were always distinct with respect to CD45 alleles, and reciprocal CD45.1/CD45.2 combinations were used as indicated in the diagram. **(B)** Peripheral blood contributions were determined by flow cytometry 5 mo after transplantation. Myeloid cells predominated in recipients of CD86^{-} HSC compared to CD86^{+} HSC ($P < 0.05$). **(C)** Absolute numbers of lymphocytes derived from recipient of CD86^{-} HSC (closed symbol) and CD86^{+} HSC (open symbol) are indicated as green (B cells), red (T cells) and blue (Myeloid cells). The data are representative of those obtained in two independent experiments (n=14). **(D)** Chimerism of donor cells in peripheral blood (PB) and bone marrow (BM) are shown as circles and triangles, respectively. Donor cells from CD86^{-} and CD86^{+} cells are indicated as closed and open symbols, respectively.

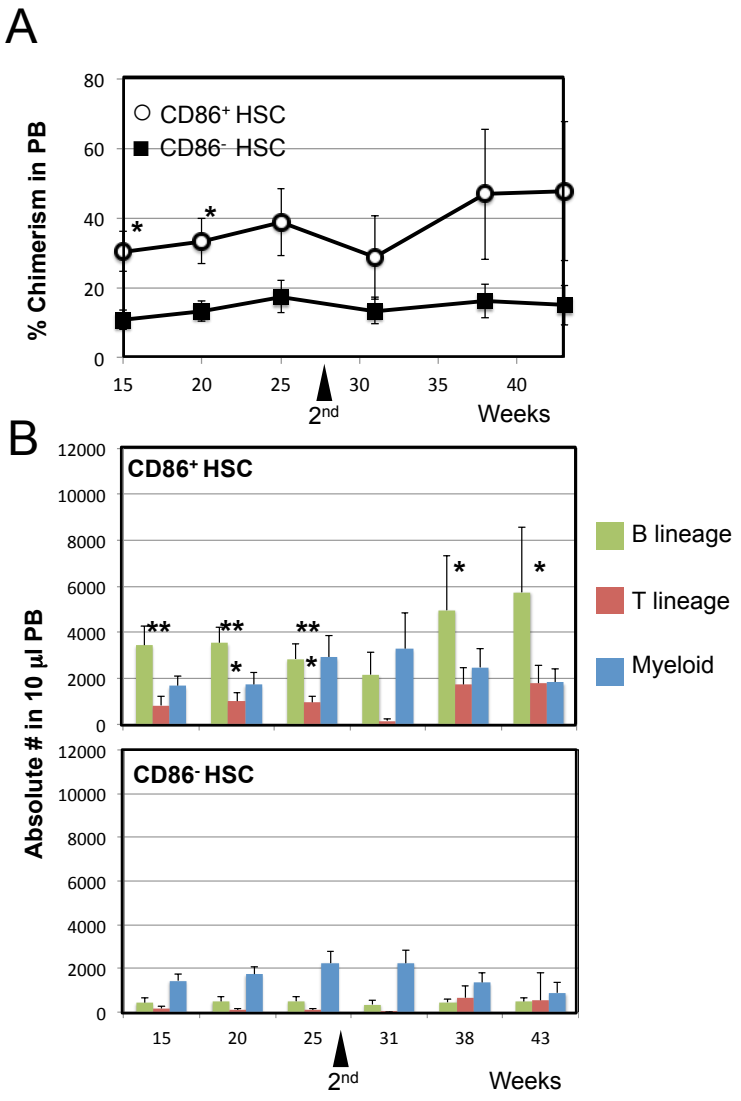

Figure S2. The poor chimerism achieved with CD86$^-$ HSC was usually not reversed by secondary transplantation and competence to produce B lineage lymphocytes remained low. **(A)** Two million whole bone marrow cells from transplanted mice (the same experiment as illustrated in Figure3) were injected into lethally irradiated recipient (CD45.1 x CD45.2 F_1) mice (marked by arrows). Peripheral blood samples were then analyzed at the indicated time points to determine donor cell chimerism. **(B)** Absolute numbers of myeloid, (GR-1 and/or CD11b), B (B220 plus CD19) or T (CD3) lineage marker bearing cells per 10 ml of blood were calculated and shown as means± SEM. Significant differences between the two groups (upper versus lower panels) are indicated (** $P < 0.01$ and *$P < 0.05$). Low CD86$^-$ derived chimerism in secondary recipients was also seen in two additional experiments where the two subsets were transplanted to the same recipients.

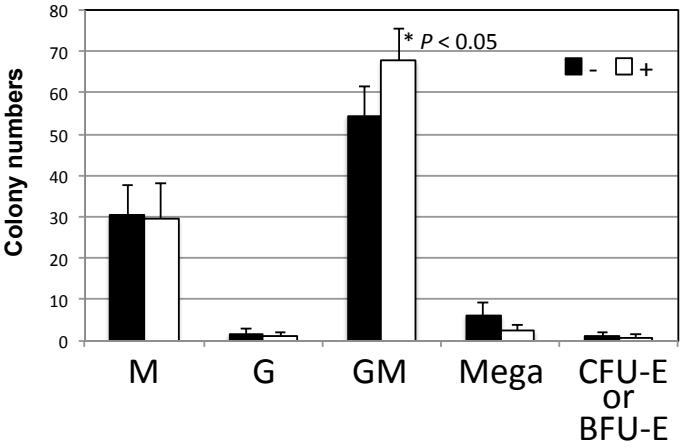

Figure S3. HSC subsets differing in CD86 expression have similar, but not identical potential to generate non-lymphoid cells. Two hundred CD150^{+} CD48^{-} Flt3^{-} cells of each type were independently sorted from three mice and cultured in Methocult medium. Colonies were counted and discriminated on the basis of morphologies 9 days later. The results are given as mean colony numbers ± SEM. (* P < 0.05). The slight difference in GM colonies was seen in two additional experiments.

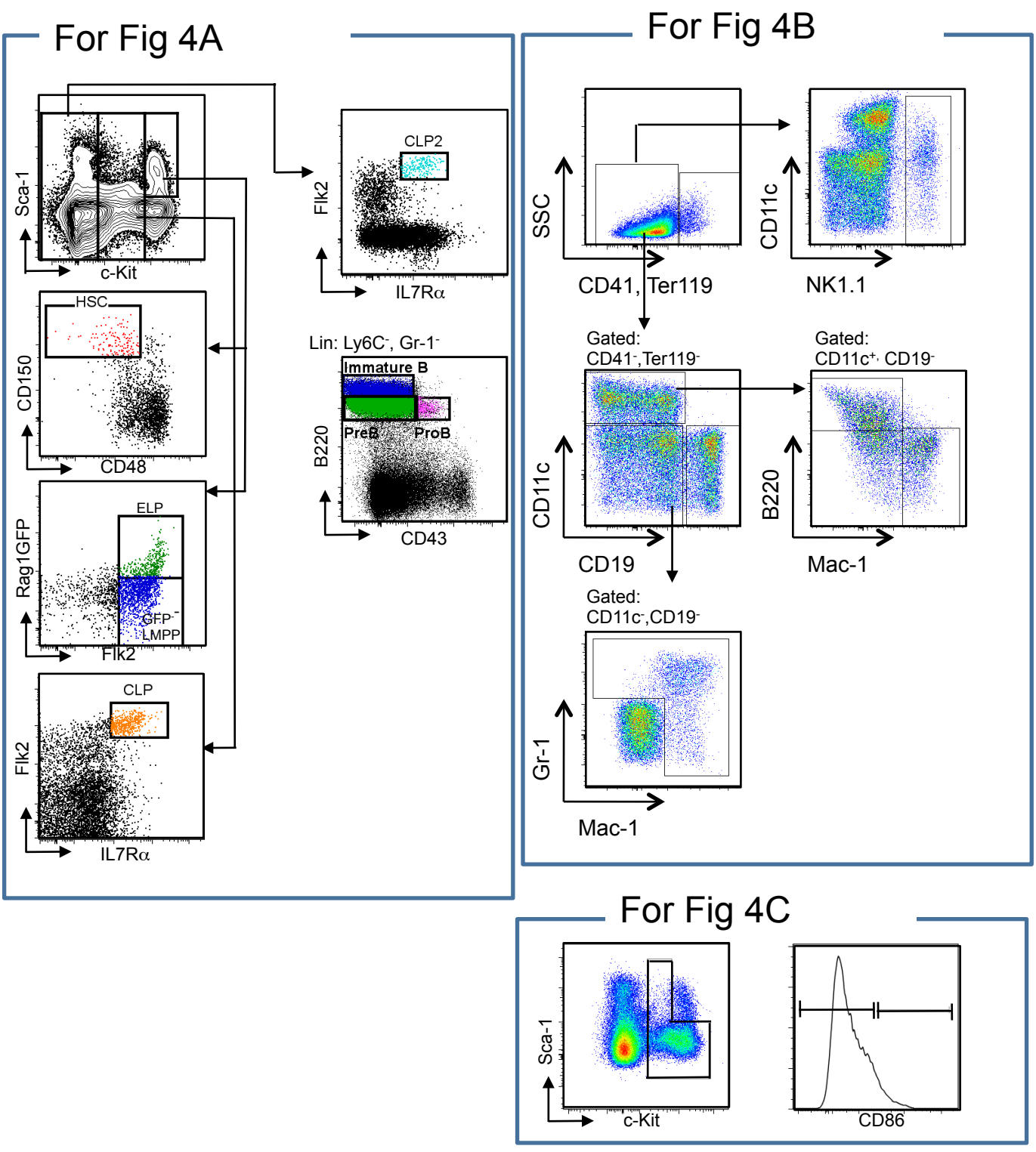

Figure S4. Resolution and characterization of CD86 expression on progenitors that were freshly isolated (corresponding to Figure 4A). Cultured cells of $CD86^{Hi}$ or $CD86^{Lo}$ LMPP were resolved according to indicated lineage specific markers(corresponding to Figure 4B). The color codes correspond to those used in the primary figures. $CD86^{+}$ and $CD86^{-}$ cells in Lin^{-} $c\text{-}Kit^{+}$ cells were sorted to specifically exclude the $Sca\text{-}1^{+}$ $c\text{-}Kit^{Hi}$ fraction (corresponding to Figure 4C).

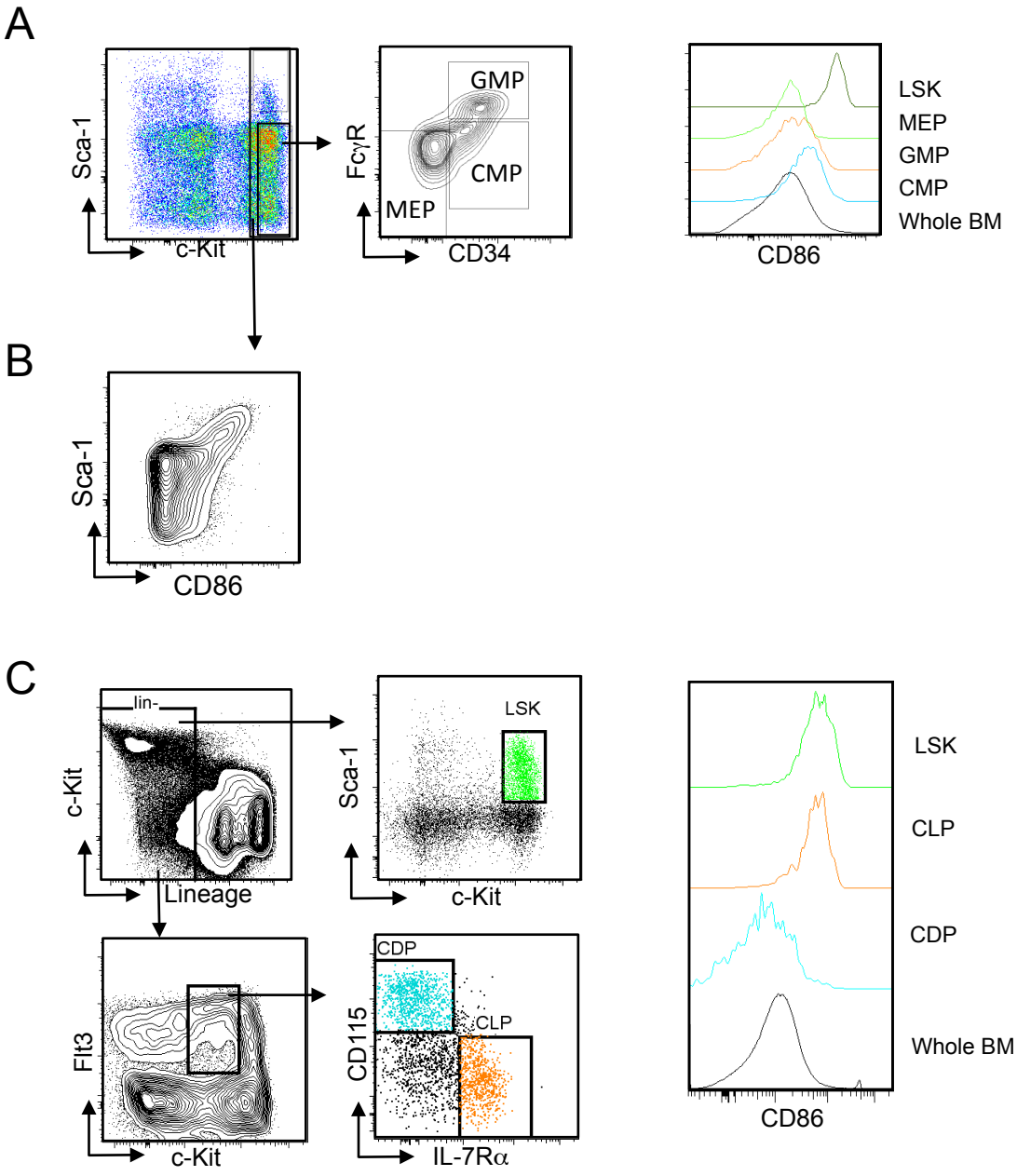

Figure S5. Demonstration of gates used to resolve hematopoietic cells and characterize their expression of CD86. **(A)** Myelo-erythroid progenitors (MEP: Lin$^-$, ckit^{Hi}, Sca-1$^-$, CD34$^-$, FcgR$^-$), common myeloid progenitors (CMP: Lin$^-$, ckit^{Hi}, Sca-1$^-$, CD34$^+$, FcgR^{Lo}), and granulocyte progenitors (GMP: Lin$^-$, ckit^{Hi}, Sca-1$^-$, CD34$^+$, FcgR$^+$), were evaluated according to the gates shown. **(B)** The stem/progenitor rich Lin$^-$ c-Kit^{Hi} marrow population was analyzed with respect to Sca-1 and CD86 expression. **(C)** Common lymphoid progenitors, but not common dendritic progenitors (CDP: Lin$^-$, ckit^{Lo}, Flt3$^+$, CD115$^+$) are CD86$^+$. LSK and whole bone marrow were used as positive and negative controls.

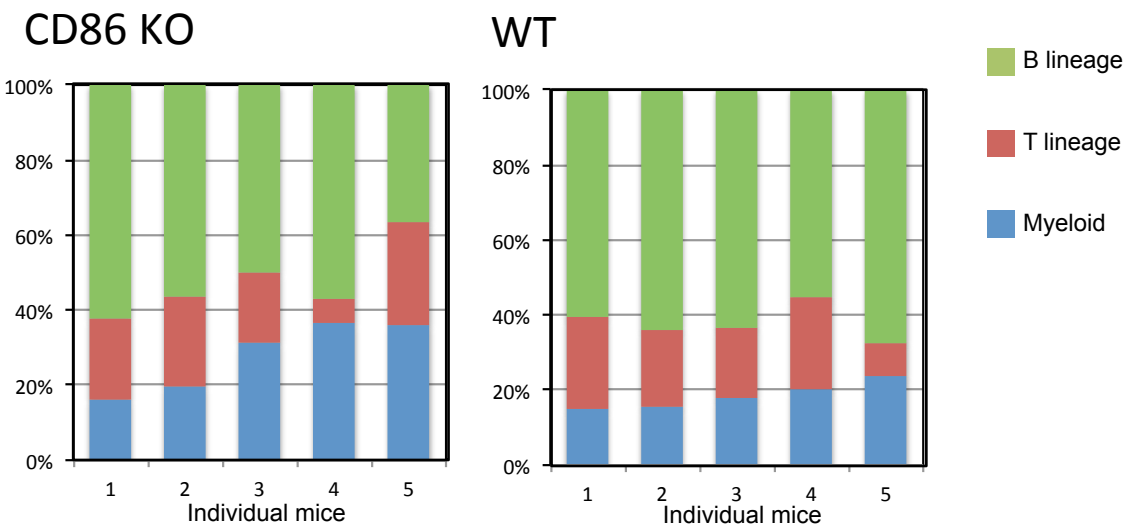

Figure S6. Hematopoiesis is not altered by targeting of the CD86 gene. Two hundred $CD150^{+}$ $CD48^{-}$ LSK from WT and CD86KO (CD45.2) animals were injected separately into recipient (CD45.1 x CD45.2 F_1) mice. Five months later, peripheral blood samples were analyzed, and no evidence for lineage skewing was found. Therefore, whole BM from the primary recipients were transplanted a second time. The bars show percentages of donor type cells that expressed myeloid, (GR-1 and/or CD11b), B (B220 plus CD19) or T (CD3) lineage markers in individual recipients five months later.

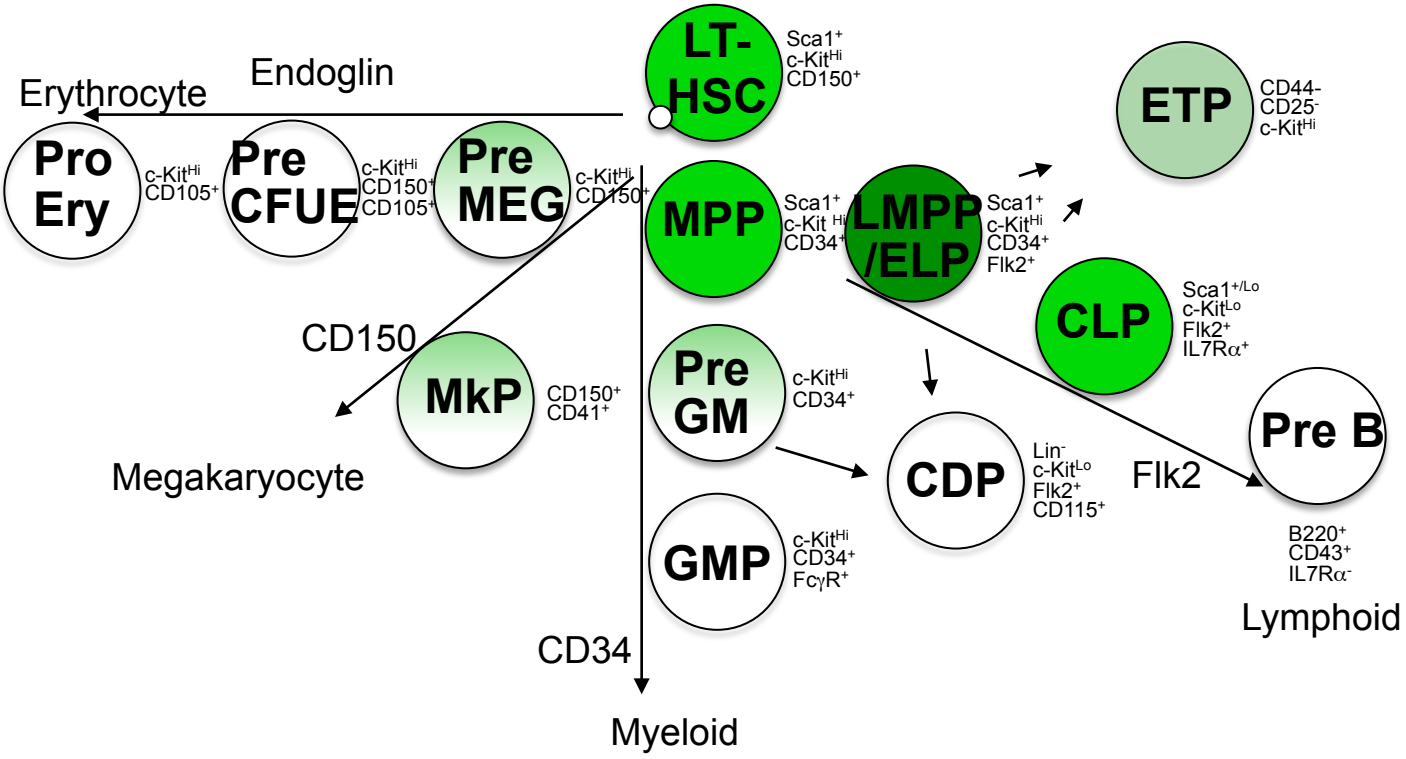

Figure S7. CD86 expression is down-regulated on progenitors destined to non-lymphoid fates. Stem and progenitor cells are arranged according to possible developmental relationships, using dark and light green shading to indicate strong and weak CD86 display, respectively. Partial shading of a cell type indicates some heterogeneity of the population, as demonstrated in primary Figures 4 and 5. While details of their resolution are given in the text, we indicate the most distinctive of characteristics for each cell type.

Supplemental Table I
Cell number of each progenitors in 10^6 BM

Cell number in 10^6 BM

	N	LSK	HSC	MPP	LMPP	preGM	preMegE	preCFU-E	proEry	CLP
WT	3	1654 ± 290	291 ± 27	526 ± 111	833 ± 153	8399 ± 43	1879 ± 470	336 ± 94	1091 ± 470	909 ± 122
CD86KO	3	1540 ± 233	282 ± 26	509 ± 111	863 ± 130	NT	NT	NT	NT	1029 ± 84