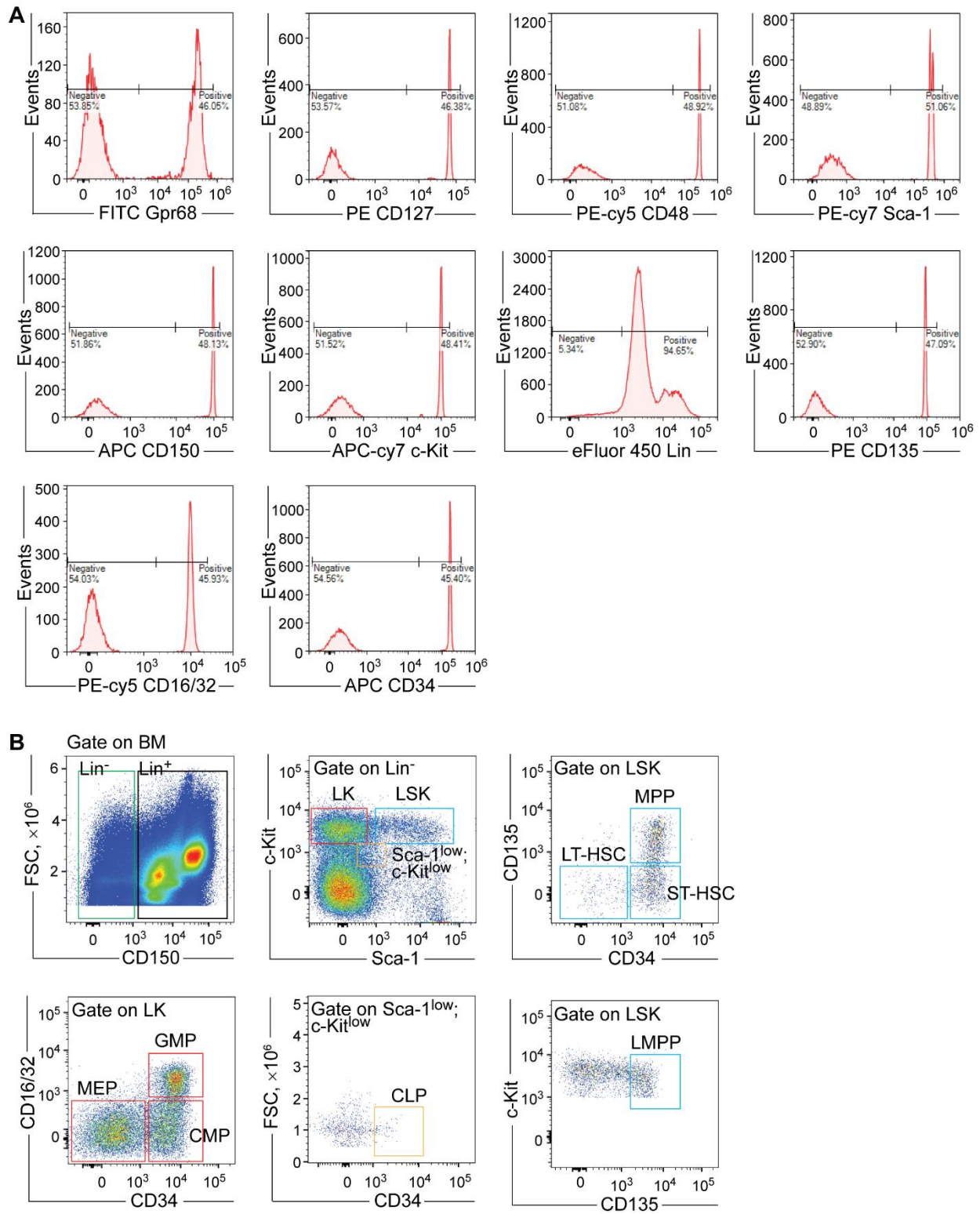


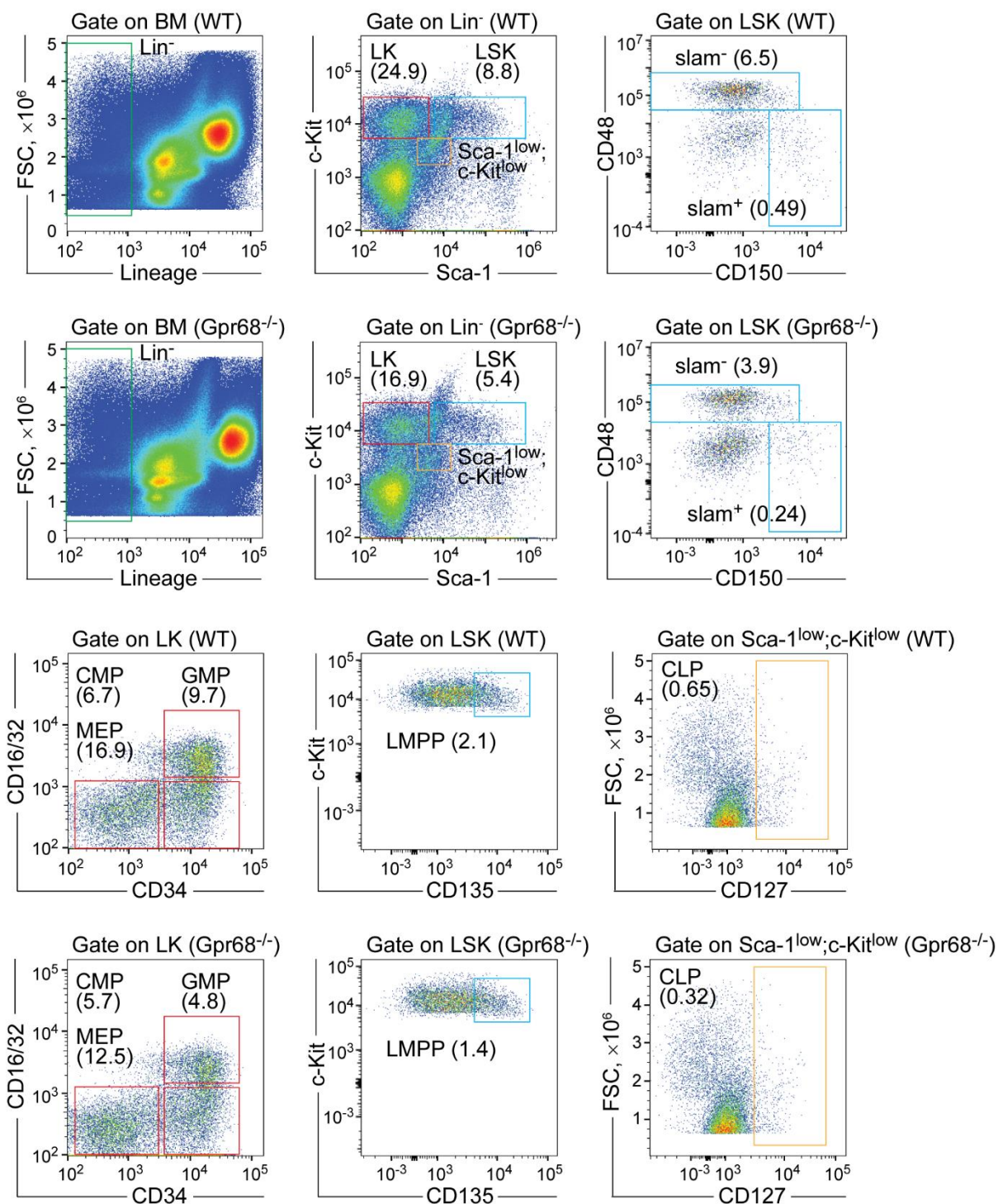
Supplemental Figure 1



Supplemental Figure 1. Gating strategy for Gpr68 expression on HSPC subpopulations.

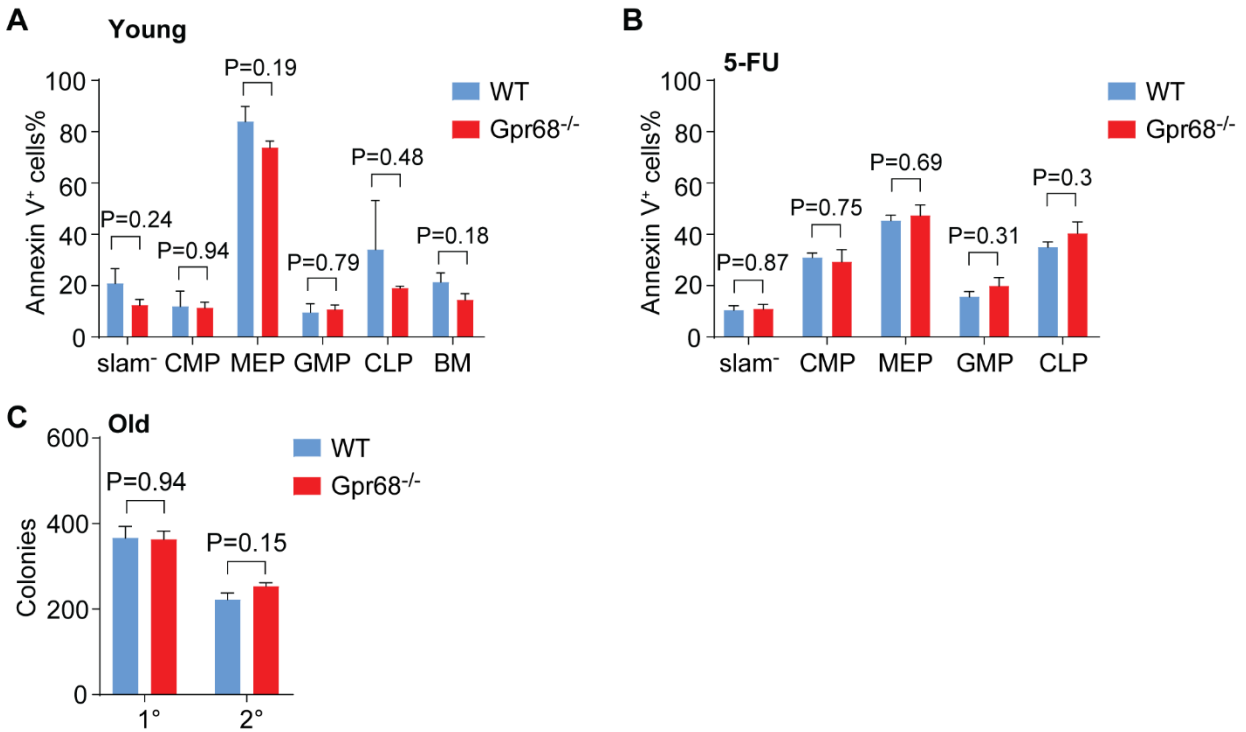
(A) Single staining control for the antibodies used to examine Gpr68 expression on HSPC subpopulations. **(B)** Gating strategy for HSPC subpopulations. Briefly, lineage⁺ and lineage⁻ cells were gated on BM MNC based on lineage markers, i.e. CD3, B220, CD11b, Gr-1, Ter119. Within the lineage⁻ BM cells, Sca-1 and c-Kit were used to identify lineage⁻, Sca-1⁺, c-Kit⁺ (LSK) cells and lineage⁻, Sca-1⁺, c-Kit⁻ (LK) cells. Within the LSK cells, CD34 and CD135 were used to identify long-term HSC (LT-HSC, lineage⁻, Sca-1⁺, c-Kit⁺, CD34⁻ and CD135⁻), short-term HSC (ST-HSC, lineage⁻, Sca-1⁺, c-Kit⁺, CD34⁺, and CD135⁻) and multipotent progenitor cells (MPP, lineage⁻, Sca-1⁺, c-Kit⁺, CD34⁺ and CD135⁺). Within the LK cells, CD34 and CD16/32 were used to identify common myeloid progenitor cells (CMP, lineage⁻, Sca-1⁻, c-Kit⁺, CD34⁺ and CD16/32⁻), megakaryocyte erythroid progenitor cells (MEP, lineage⁻, Sca-1⁻, c-Kit⁺, CD34⁻ and CD16/32⁻) and granulocyte monocyte progenitor cells (GMP, lineage⁻, Sca-1⁻, c-Kit⁺, CD34⁺ and CD16/32⁺). Lymphoid-primed multipotent progenitor cells (LMPP) were identified as lineage⁻, Sca-1⁺, c-Kit⁺ and CD135⁺. Common lymphoid progenitor cells (CLP) were identified as lineage⁻, Sca-1^{low}, c-Kit^{low} and CD127⁺.

Supplemental Figure 2



Supplemental Figure 2. Numbers of HSPC in BM from WT and *Gpr68*^{-/-} mice. Representative flow cytometric analysis of HSPC in BM from WT and *Gpr68*^{-/-} mice (4~5 months old).

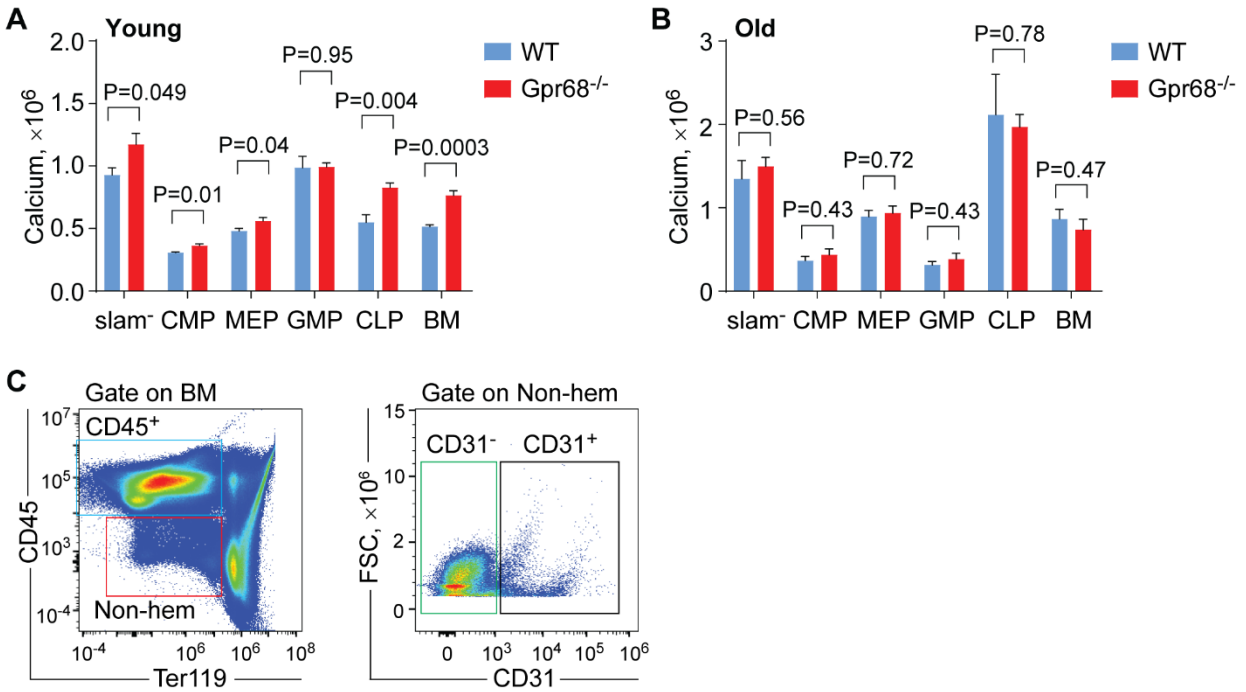
Supplemental Figure 3



Supplemental Figure 3. Cellular features are not altered in HSC from Gpr68^{-/-} mice. (A-B)

Frequency of Annexin V⁺ cells in HSPC and BM lineage⁺ cells from WT and Gpr68^{-/-} mice at young age (4~5 months old, n=3, A) and post 5-FU injection (8 months old, n=5, B). (C) Numbers of colonies formed by BM MNC from WT and Gpr68^{-/-} mice after primary (1°) and secondary (2°) replating (12 months old, n=3).

Supplemental Figure 4



Supplemental Figure 4. Signaling pathways in HSPC from Gpr68^{-/-} mice. (A-B) Cytosolic Ca²⁺ levels in HSPC subpopulations from WT and Gpr68^{-/-} mice at young age (4~5 months old, n=5, A) and upon aging (10~11 months old, n=5, B). **(C)** Flow cytometric analysis of hematopoietic cells (CD45⁺), non-hematopoietic cells (CD45⁻ and Ter119⁻), CD31⁺ and CD31⁻ cells in BM from C57Bl6 mice.