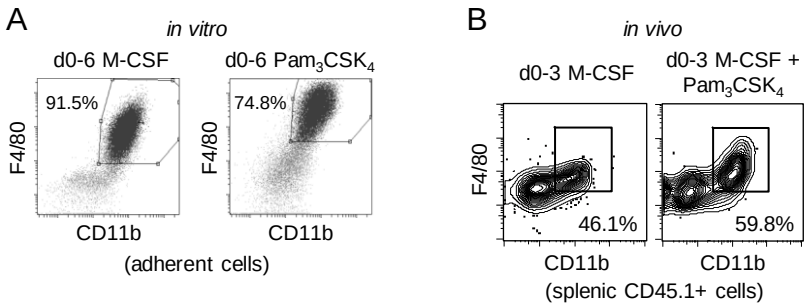
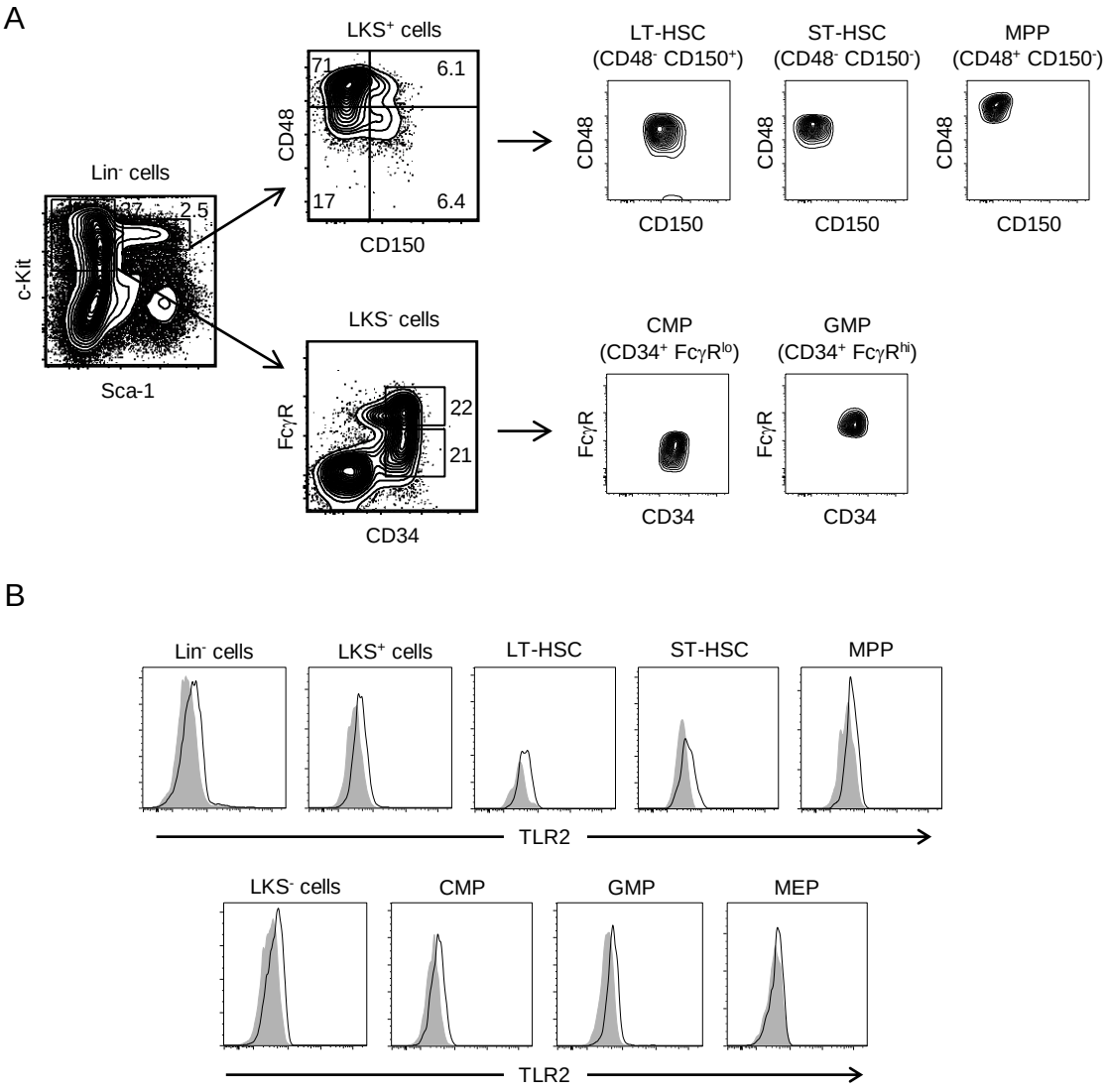
Supplementary Figure 1



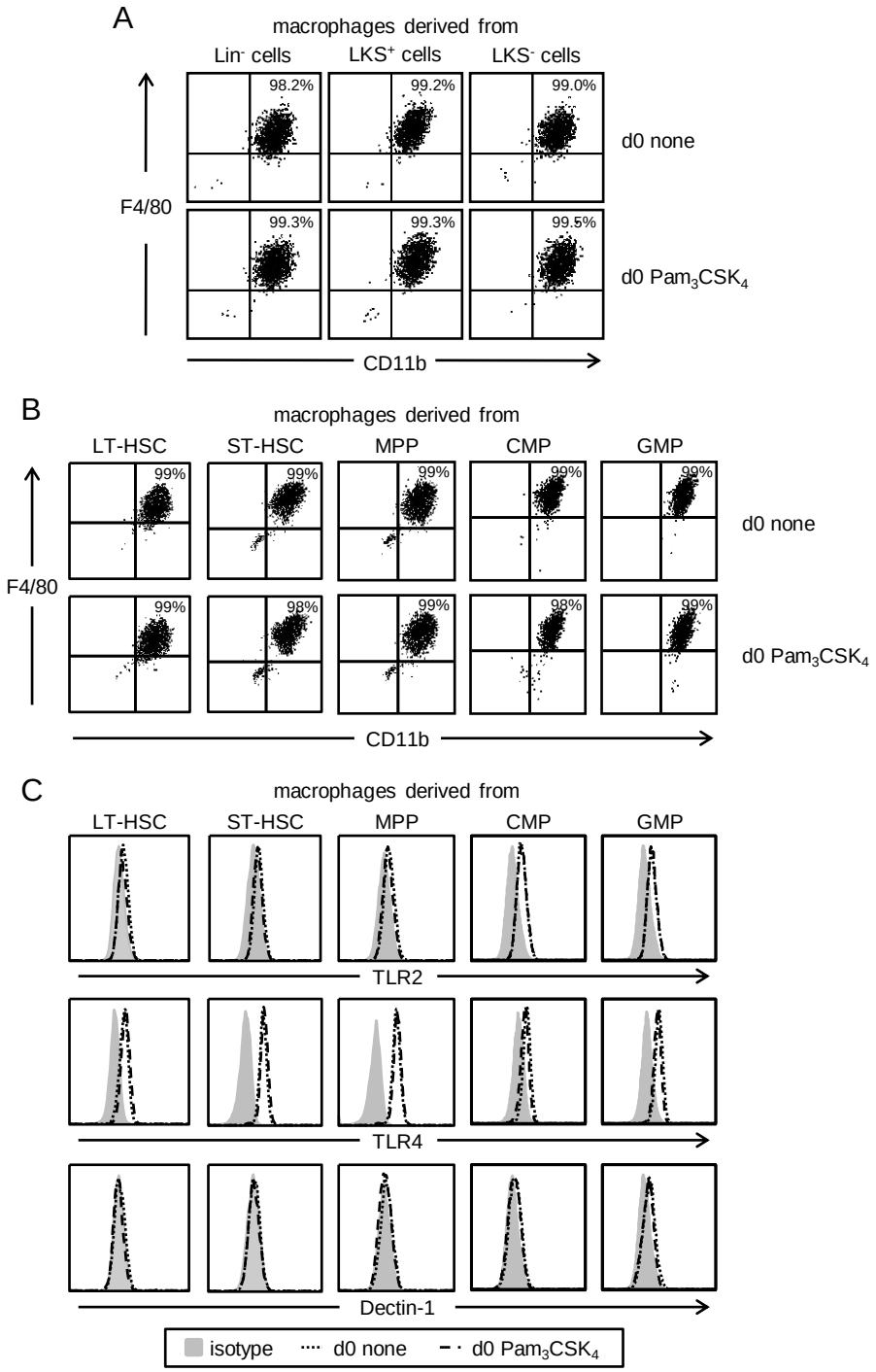
Supplementary Figure 1. Macrophage marker expression by macrophages derived in the presence of Pam_3CSK_4. (A) Lin- HSPCs from mouse bone marrow were cultured for 6 days in media containing serum plus SCF and Flt3L, and 50 ng/ml M-CSF or 1 μg/ml Pam_3CSK_4 (d0-6 M-CSF and d0-6 Pam_3CSK_4 respectively). Adherent cells were harvested for analysis of surface expression of CD11b and F4/80 by flow cytometry. Data are representative of 3 independent experiments. (B) HSPCs from wild type CD45.1 donor mice were intravenously injected into TLR2 -/- CD45.2 recipient mice. Recipient mice then received an injection of 10 μg M-CSF plus/minus 100 μg Pam_3CSK_4 on days 0, 1 and 2. Spleens were harvested on day 3 and surface expression of CD11b and F4/80 by donor-derived CD45.1+ cells was assessed by flow cytometry. Data are representative of 3 mice/group.

Supplementary Figure 2



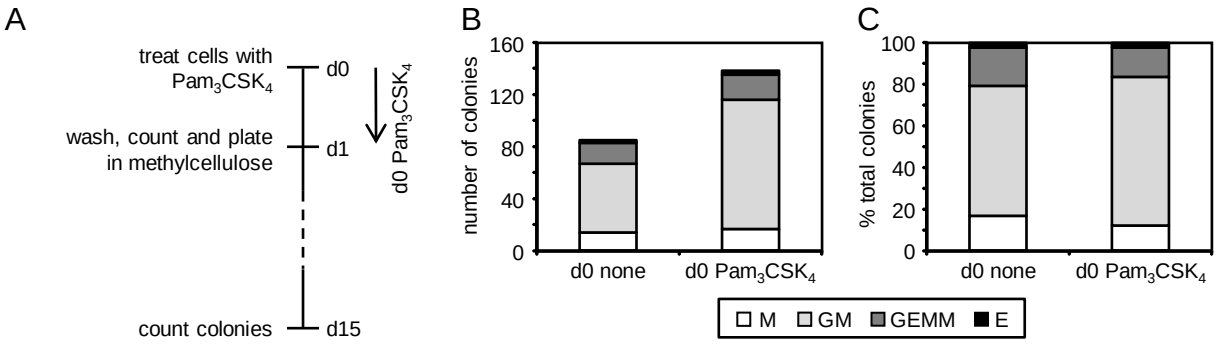
Supplementary Figure 2. HSPC sorting strategy and surface expression of TLR2 by HSPC subsets. (A) HSPC subsets were sorted from mouse bone marrow by first depleting Lin^+ cells by MACS, and then FACS sorting according to surface expression of stem/progenitor markers. (B) Surface expression of TLR2 by mouse HSPC subsets was assessed by flow cytometry. Data are representative of at least 3 independent experiments.

Supplementary Figure 3



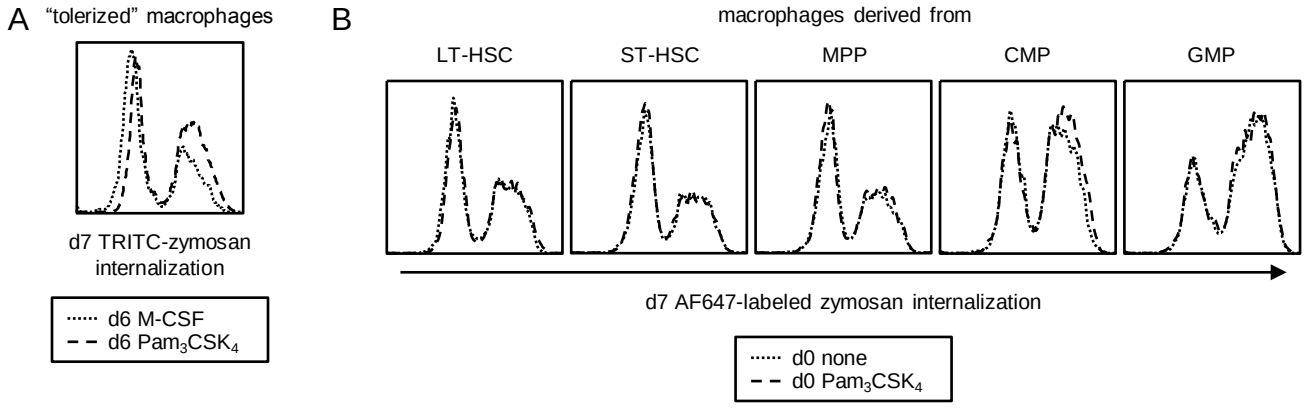
Supplementary Figure 3. Macrophage marker and pattern recognition receptor expression by macrophages derived from Pam_3CSK_4-treated HSPC subsets. Mouse HSPCs were treated with 100 ng/ml Pam_3CSK_4 for 24 h, washed, and cultured with M-CSF (plus SCF and Flt3L for LT-HSC, ST-HSC and MPP cultures) for a further 5 (Lin-, CMP, GMP), 12 (LKS+, LKS-, MPP, ST-HSC) or 14 (LT-HSC) days to derive macrophages. Macrophage marker (A and B) and pattern recognition receptor (C) expression was assessed by flow cytometry. Data are representative of 3 independent experiments.

Supplementary Figure 4



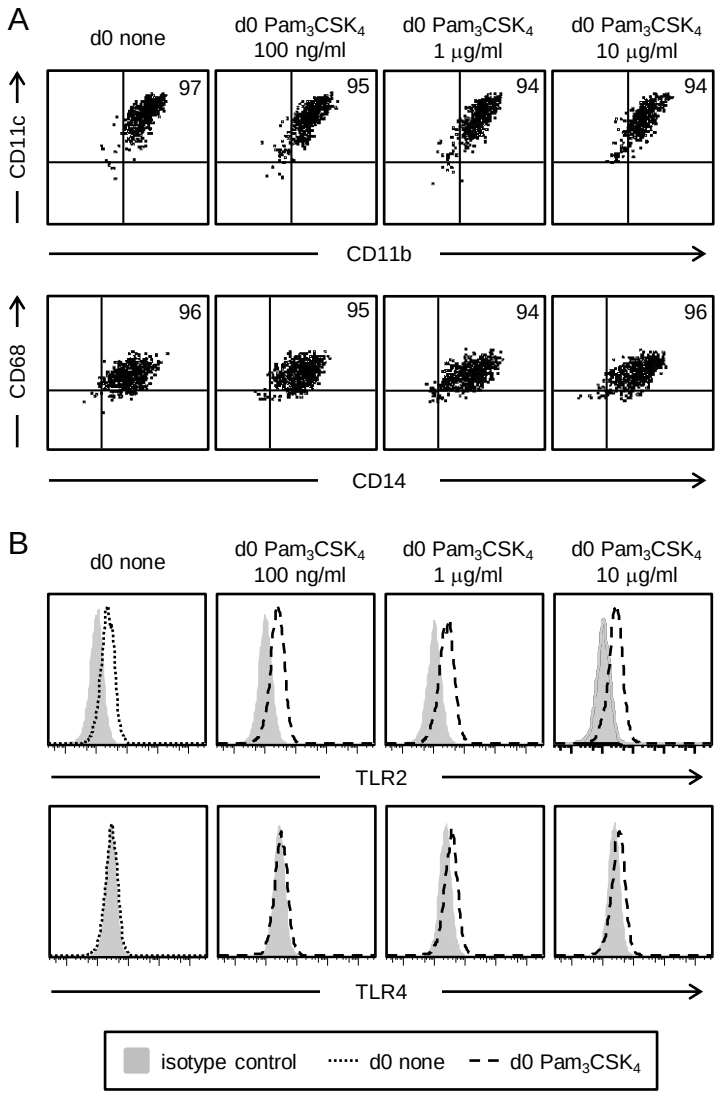
Supplementary Figure 4. Effect of transient exposure of murine HSPCs to a TLR2 agonist on myeloid colony formation in methylcellulose. (A) d0 Pam_3CSK_4-exposed mouse Lin- cells were washed and plated in quadruplicate (1 x 10^3 cells/well) in methylcellulose. Day 15 colonies are presented as colony number (B) and percentage of total colonies (C). M – monocyte, GM – granulocyte/monocyte, GEMM – granulocyte/erythrocyte/monocyte/megakaryocyte, E – erythrocyte colonies. Representative data are shown. Data are representative of 3 independent experiments.

Supplementary Figure 5



Supplementary Figure 5. Phagocytic capacity of macrophages derived from Pam_3CSK_4-exposed HSPC subsets. (A) Macrophages derived from mouse Lin- cells using M-CSF were treated on day 6 with 100 ng/ml Pam_3CSK_4 and washed on day 7 prior to assessment of phagocytosis of 20 μg/ml fluorescently-labeled zymosan (15 min stimulation) by flow cytometry. (B) Mouse HSPCs were treated with 100 ng/ml Pam_3CSK_4 for 24 h, and thoroughly washed prior to culture with M-CSF (plus SCF and Flt3L for LT-HSC, ST-HSC and MPP cultures) for a further 5 (CMP, GMP), 12 (MPP, ST-HSC) or 14 (LT-HSC) days to derive macrophages. Macrophages were plated at equal numbers and rested overnight prior to assessment of phagocytosis of 20 μg/ml fluorescently-labeled zymosan (15 min stimulation). Data are representative of at least 3 independent experiments.

Supplementary Figure 6



Supplementary Figure 6. Macrophage marker expression by macrophages derived from human HSPCs transiently exposed to a TLR2 agonist prior to differentiation. Human CD34+ cord blood cells were treated with Pam_3CSK_4 at the indicated doses for 24 h, and then thoroughly washed prior to culture with M-CSF for 3 weeks to derive macrophages. Macrophage marker expression was assessed by flow cytometry on day 22. Data are representative of 3 independent experiments.