SUPPLEMENTAL FIGURES

Supplemental Figure 1. Fluorescence-activating cell sorting and monocyte functional assays.

A) Shows an example of bone marrow monocyte progenitors isolated by FACS to an initial purity of approximately 97%. A second sort was necessary to reach 100% purity. Single cell sorting of populations PIV, PV and PVI were performed to test the differentiation and functionality of macrophages, dendritic cells and osteoclasts. B) Peripheral monocytes were purified using the same approach shown in A). The FACS dot plots shown are of peripheral blood monocytes. Single cell sorting for both spleen and peripheral blood monocytes was performed only to test the ability of these cells to differentiate to macrophages, dendritic cells and osteoclasts, functional assays required a higher number of cells.

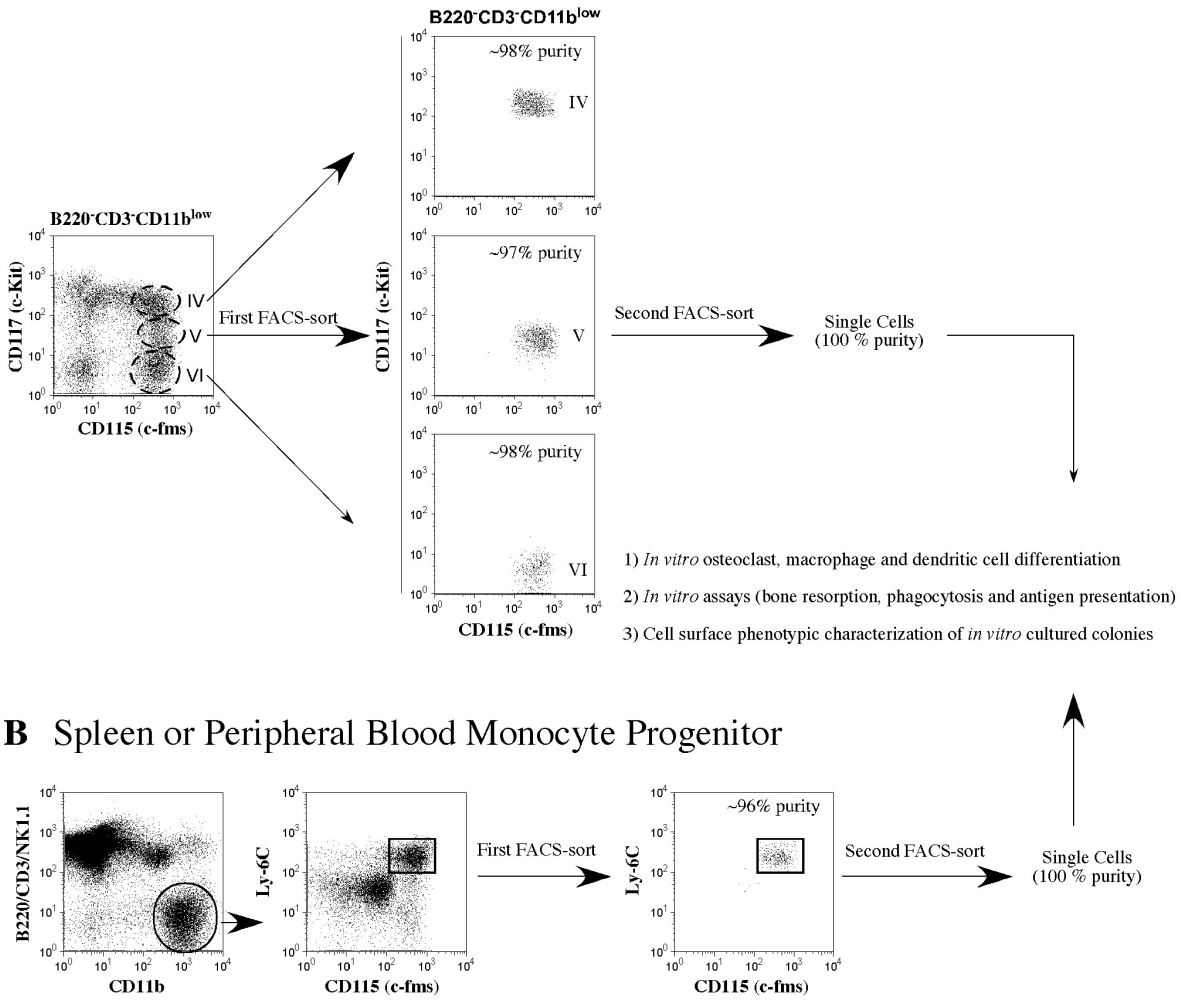
Supplemental Figure 2. Single cell sorting of peripheral tissue monocyte progenitors.

A) Spleen or B) peripheral blood monocyte progenitors were single cell sorted by FACS to a density of 1, 5, 50 or 100 cells per well, and tested for their ability to differentiate to macrophages, dendritic cells and osteoclasts. Single cell sorts of 1, 5, 50 or 100 cells per well of spleen monocytes were cultured for 13 days with M-CSF for macrophages (left bar graph), 13 days with M-CSF and 7 additional days with both M-CSF and RANKL for osteoclasts (middle bar graph) or 13 days with GM-CSF and 7 additional days with both GM-CSF and IL-4 for dendritic cells (right graph). For peripheral blood monocytes, single cell sorts of 1, 5, 50 or 100 cells per well were cultured for 15 days with M-CSF for macrophages (left bar graph), 15 days with M-CSF and 5 additional days with both M-CSF and RANKL for osteoclasts (middle bar graph) or 14 days with GM-CSF and 6 additional days with both GM-CSF and IL-4 for dendritic cells (right graph).

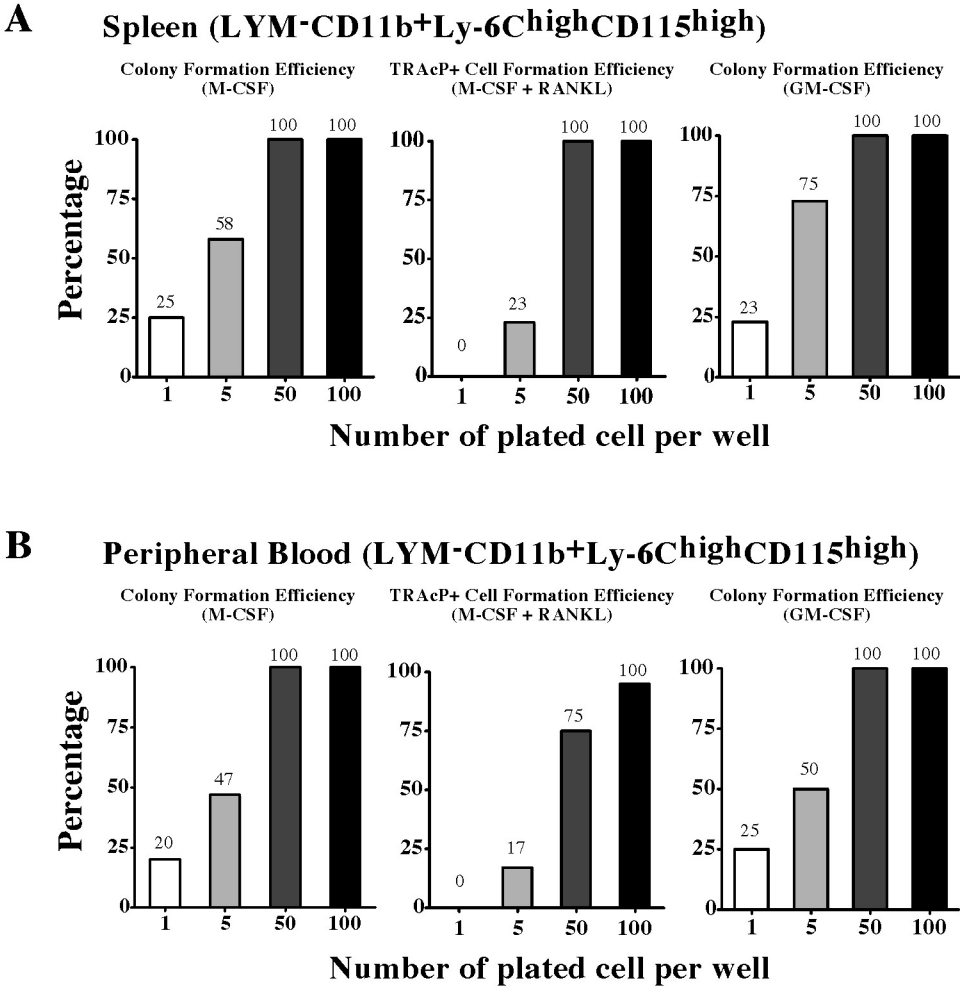
Supplemental Table 1. Phenotypic comparison of bone marrow and peripheral tissue monocyte progenitors.

Comparison of cell surface markers expression of freshly isolated monocyte progenitors derived from bone marrow, spleen or peripheral blood. Differences found among progenitors are highlighted.

Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Table 1.

	BM (PIV)	Spleen	PBL
B220	(-)	(-)	(-)
CD3	(-)	(-)	(-)
NK1.1	(-)	(-)	(-)
CD11b	low	high	high
Ly-6C	high	high	high
Ly-6G	(-)	(-)	(-)
CD115	high	high	high
CD117	high	low	low
CD11c	low	low/high	low
F4/80	(+)	(+)	(+)
CD14	(+)	(+)	(+)
TLR4	(+)	(+)	(+)
CX3CR1	low	low	low
CD62L	low	low	low
CCR2	low	high	high
MHCI	(+)	(+)	(+)
MHCII	low	low	low
CD40	low	low	(-)
CD80	low	high	low
CD86	low	low	low

Bone marrow monocyte: **B220^{-}CD3^{-}CD11b^{lo}CD117^{high}CD115^{high}**
Spleen monocyte: **LYM^{-}CD11b^{high}Ly-6C^{high}CD115^{high}**
Peripheral blood monocyte: **LYM^{-}CD11b^{high}Ly-6C^{high}CD115^{high}**