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Supplementary

Impact of Erythropoietin Production by Erythroblastic Island Macrophages on Homeostatic Murine Erythropoiesis

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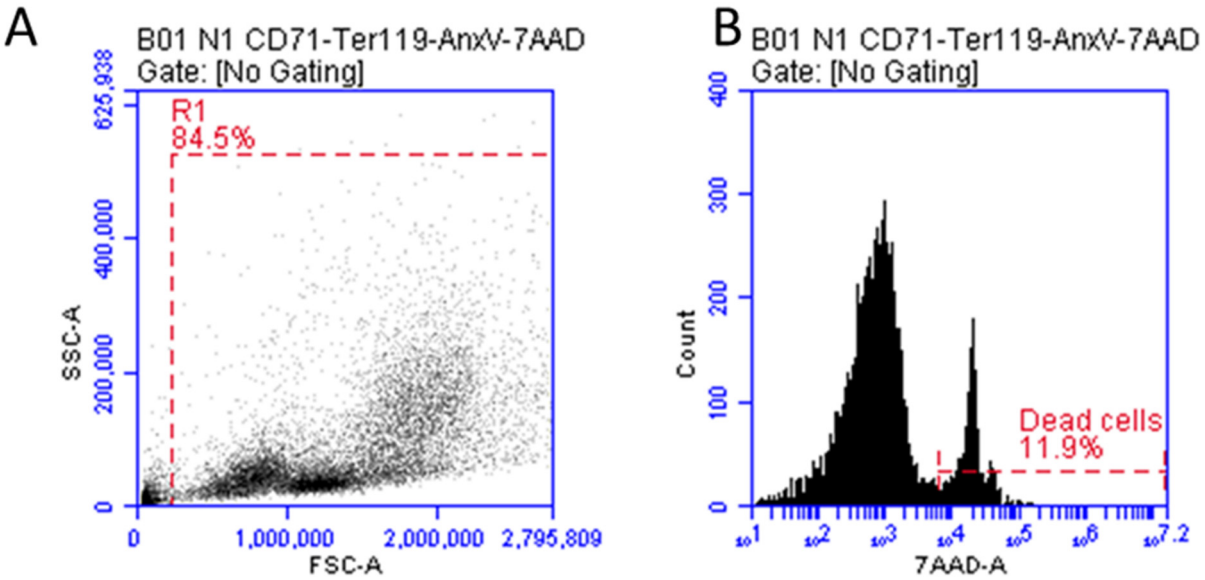

Figure 1. Gating strategies for the exclusion of debris and dead cells. (**A**) A gate was created broadly to include cells but exclude debris. For each sample, 10 000 events were collected inside this gate. (**B**) 7AAD+ cells were selected on a histogram plot for exclusion in later analysis.

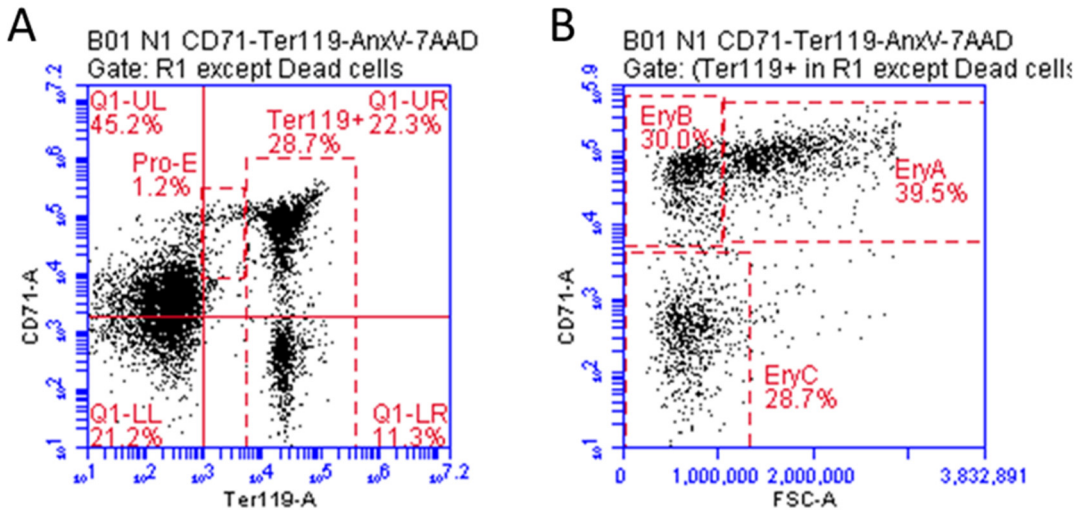

Figure 2. Gating strategies for identification of ProE, EryA, EryB and EryC. (**A**) Live cells were analyzed by expression of Ter119 and CD71. $CD71^{high}Ter119^{med}$ cells were identified as proerythroblasts (ProE). A gate was created to select all $Ter119^{high}$ cells. (**B**) $Ter119^{high}$ cells were analyzed by size (FSC) and expression of CD71. EryA were identified as $CD71^{high}FSC^{high}$, EryB as $CD71^{high}FSC^{low}$ and EryC as $CD71^{low}FSC^{low}$.

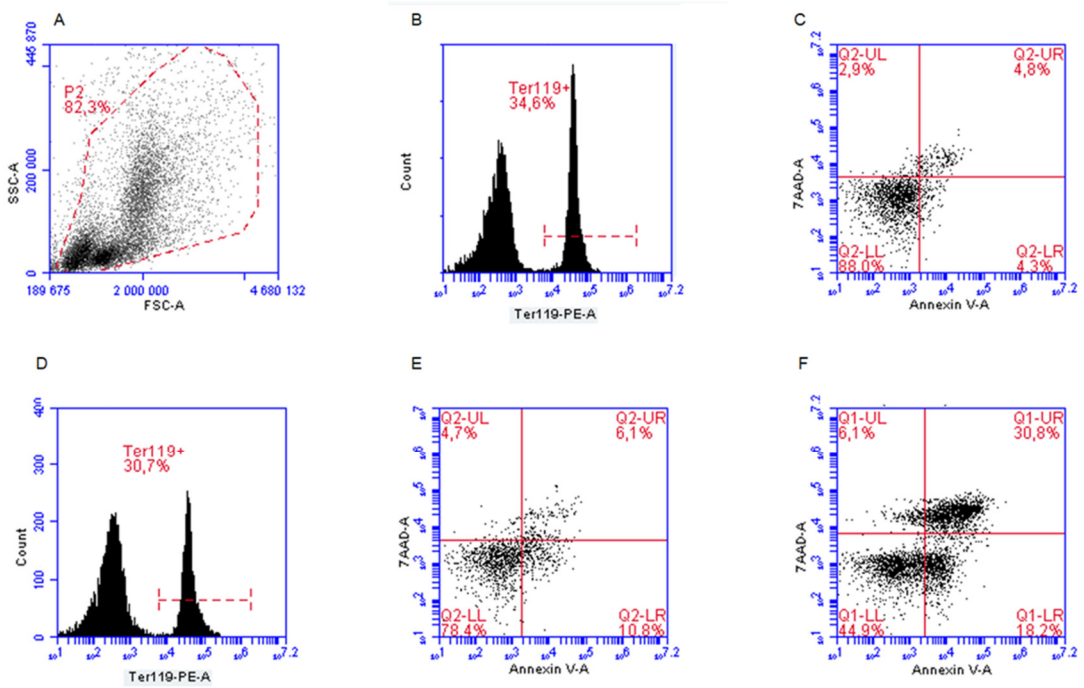

Figure 3. Strategy to evaluate apoptotic cells in the bone marrow and spleen by flow cytometry. Bone marrow and splenic cells were stained with anti Ter119 antibody, Annexin V and 7AAD. (**A**) Gate to exclude debris. (**B**) Selection of Ter119+ cells in the BM and (**C**) assessment of Annexin V+ and Annexin V+7AAD+ cells within BM Ter119+ cells. (**D**) Selection of Ter119+ in the spleen and (**E**) analysis the percentages of annexin V+ and annexinV+7AAD+ cells within Ter119+ cells in the spleen. (**F**) Splenic apoptotic erythroblasts following 48 hours in culture; supernatants from these cells were used to condition BMDM prior to their co-culture with LSK cells for evaluation of pro-erythropoietic activity.

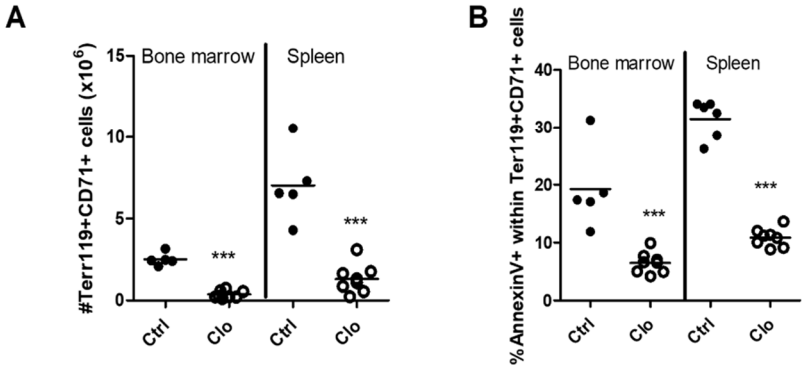

Figure 4. Assessment of total Ter119+ CD71+ cells and apoptosis levels in control mice and in mice treated with clodronate liposomes. (A) Compilation of Ter119+CD71+ cell numbers in the BM and spleen from control (Ctrl) and clodronate -treated (Clo) mice one week after injection of liposomes; significantly lower numbers of Ter119+ cells are found in mice ablated of macrophages. (B) The percentages of apoptotic annexin V+ Ter119+Cd71+ cells significantly decreased in the BM and spleen from clodronate treated mice. *** $p<0.001$.