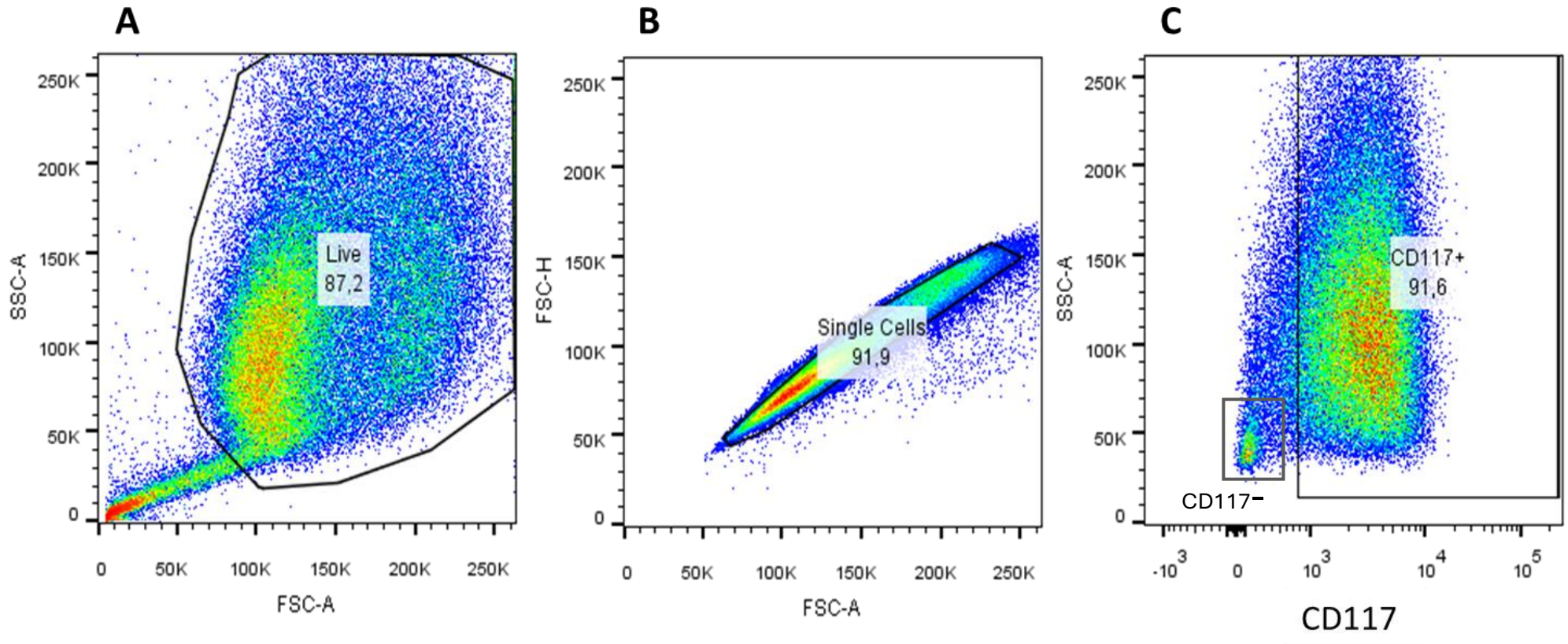
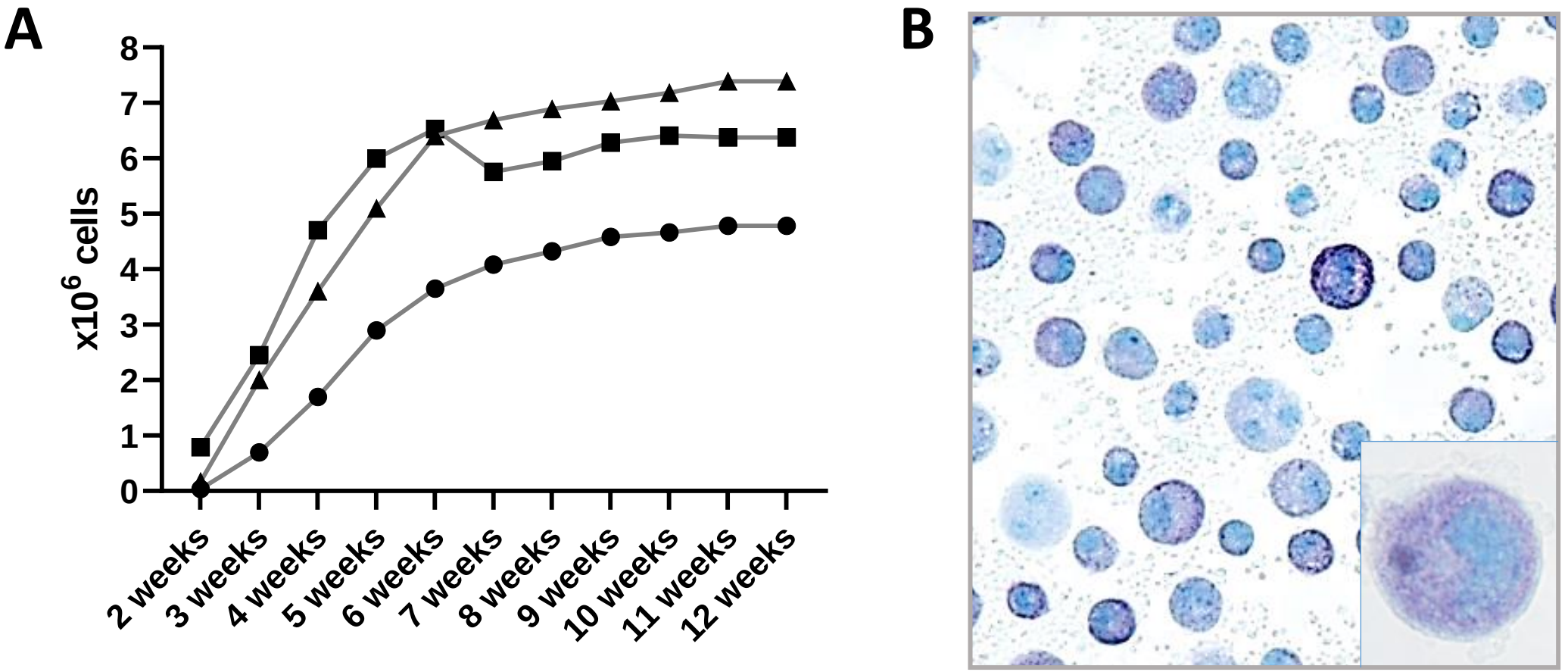
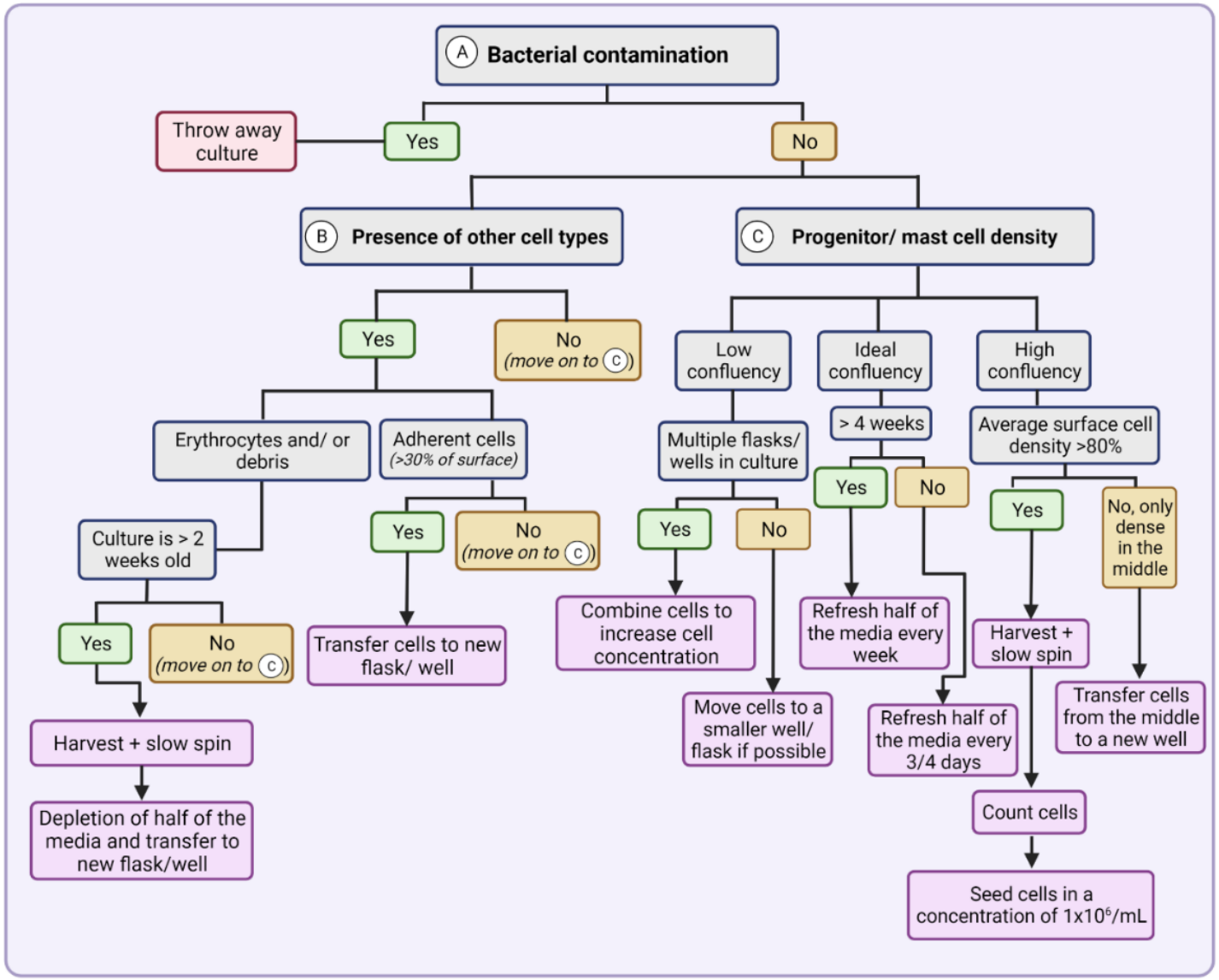


Supplemental Figure S1 Gating strategy prior to assessment of cell surface expression by mature MC obtained after 12 weeks of culture
Cells were first gated based on FSC/SSC profile (A) whereafter doublets were removed based on FSC-A/FSC-H gating as shown in (B) prior to assessment of % cells expressing CD117 (C). The small $CD117^{neg}$ population shown in (C) served as a background antibody staining control. Data analysed with FlowJo Software version 10.8.1



Supplemental Figure S2 Absolute counts and morphology of nucleated cells generated from a single venous blood sample (A) Absolute cell numbers (Y-axis) of live nucleated cells assessed in different cultures set up with HSPC isolated from different volumes of peripheral blood drawn at the same time from one healthy adult donor, being 35 ml (filled squares), 21 ml (filled triangles) or 14 ml of blood (filled circles). Graph created with GraphPad Prism. (B) Toluidine blue staining showing heterogeneity in size and granule content of PB-MC derived from healthy donor 3 after 12 weeks of culture. Insert shows a higher power magnification.



Supplemental figure S3 Decision tree for the generation of MC from peripheral blood

The scheme provides an overview of key steps to follow when generating MC from small volumes of human PB. Culture characteristics to check are: (A) bacterial contamination, (B) presence of other cell types, and (C) HSPC/mature mast cell density. Figure created with BioRender.com