Supplementary Material

A microwell device for the efficient generation of arrays of microtissues and humanized bone marrow micro-ossicles

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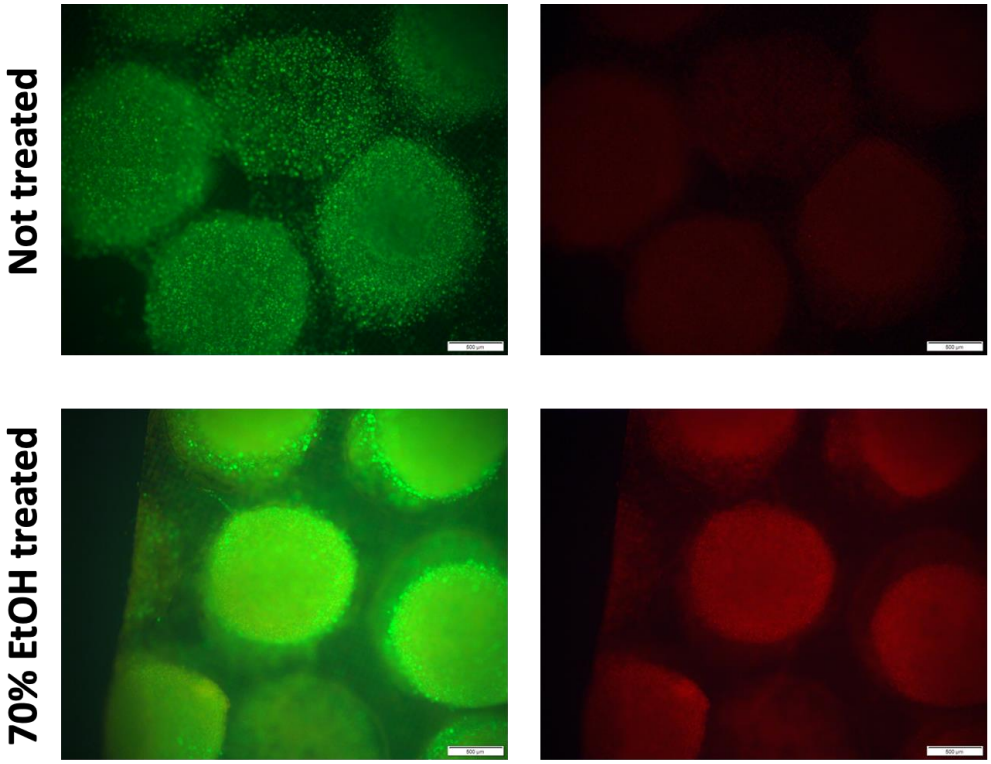

Figure S1. Dead cell control for Live/Dead viability characterization. Dead cell control tissues were first incubated for 30 minutes in 70% methanol, and then submerged in 2 μM calcein AM and 4 μM of ethidium homodimer-1 in PBS and incubated in the dark at room temperature for 45 minutes. Red indicates dead cells. Scale bars = 500 um.

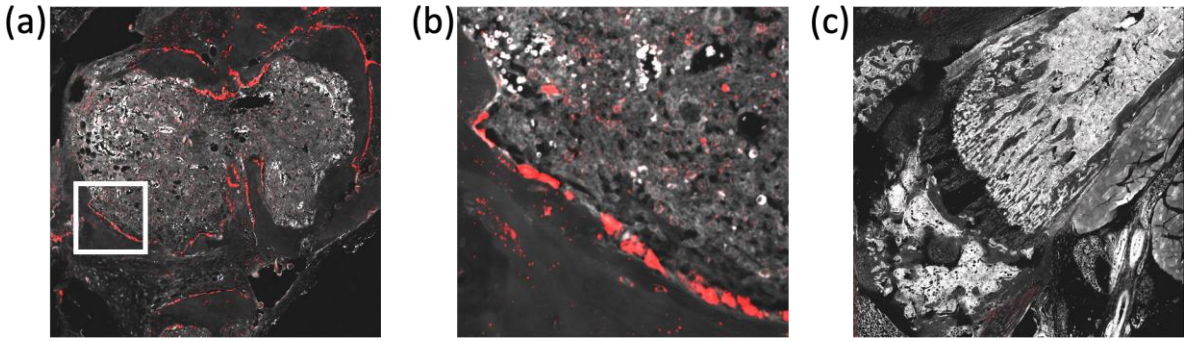

Figure S2. Human GAPDH (hGAPDH) staining in tissue with RNAscope (red). **(a)** hGAPDH staining in week 16 micro-ossicles transplanted with human $CD34^+$ cells. hGAPDH-stained cells in the marrow and in the bone tissue. **(b)** Zoomed-in image of white box area from (a). **(c)** Cross section of mouse femur at synovial joint, not transplanted with human $CD34^+$ cells. No hGAPDH-stained cells. White is cell/tissue autofluorescence.

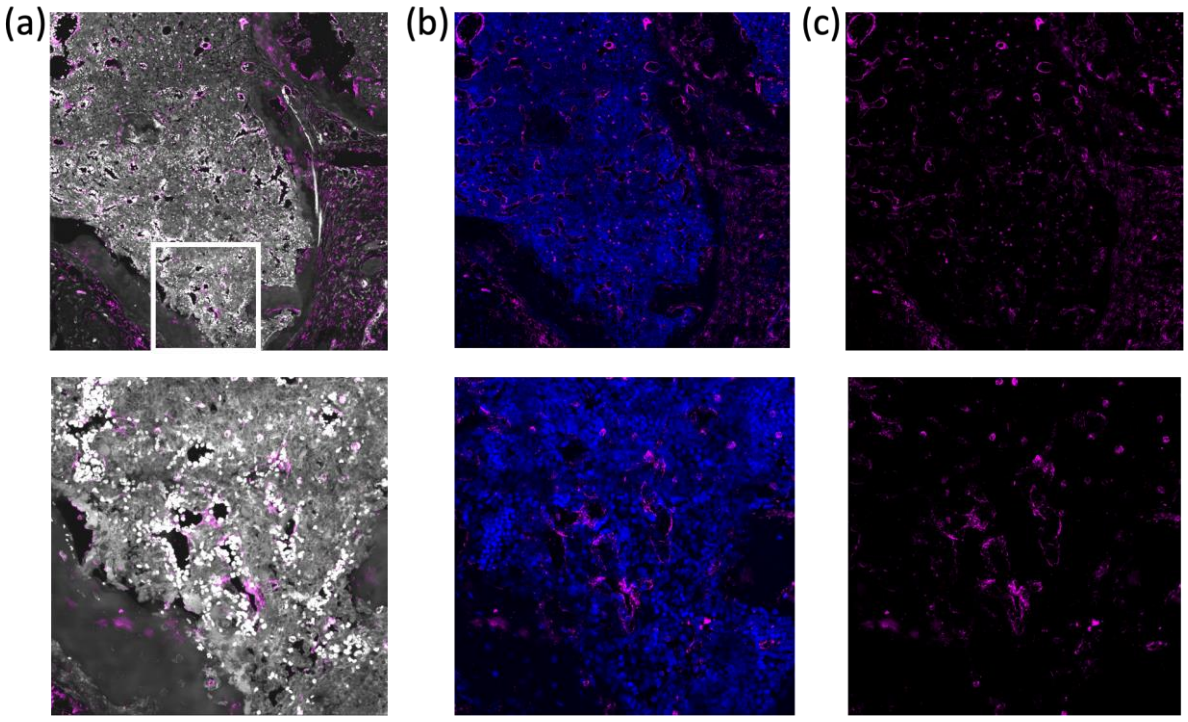

Figure S3. Micro-ossicles stained with rat anti-mouse CD13 and rat anti-mouse Endomucin). **(a)** Tissue autofluorescence (white) and anti-CD31/anti-Endomucin (magenta) localized to vascular structured and fibrous tissue, **(b)** without autofluorescence, but with DAPI (blue) and (c) anti-CD31/anti-Endomucin (magenta) channel only. Upper panel is 40x images stitched/tiled images, and lower panel is zoomed in on white box area from upper panel. Note that very bright [white] cells are autofluorescent red blood cells.

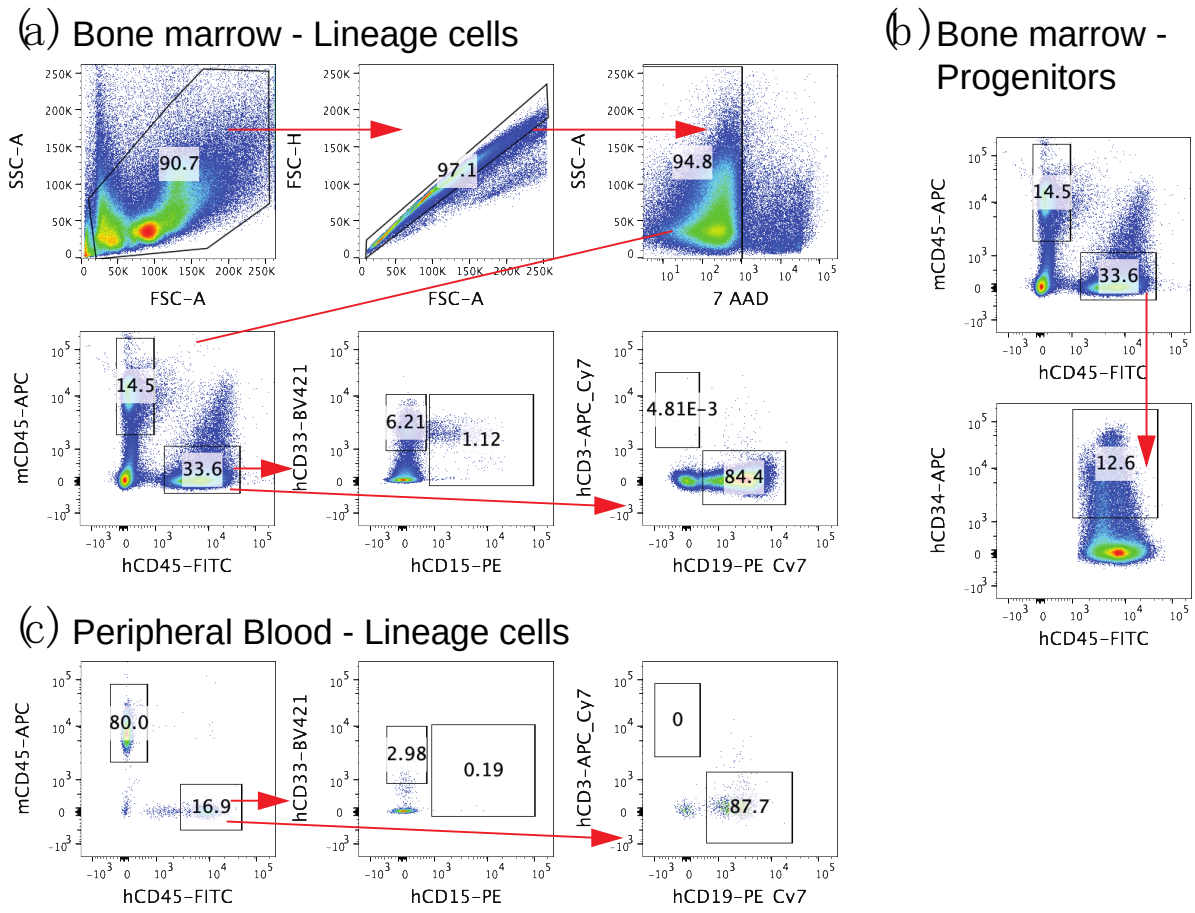

Figure S4. Gating strategy for flow cytometry analysis of human hematopoietic cells in mouse bone marrow, micro-ossicles and in peripheral blood. **(a)** bone marrow lineage cells, **(b)** bone marrow progenitor cells, and **(c)** peripheral blood lineage cells.