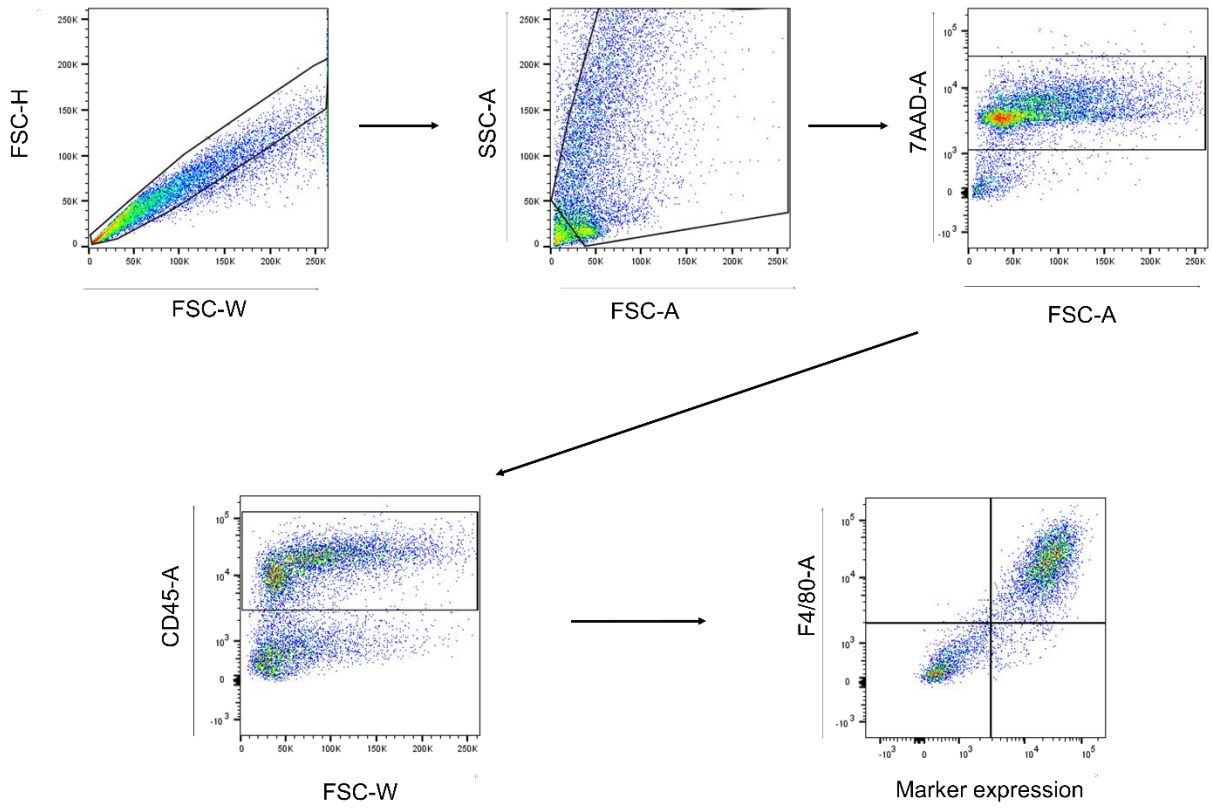
ONLINE APPENDIX

Multinucleated giant cells in adipose tissue are specialized in adipocyte degradation

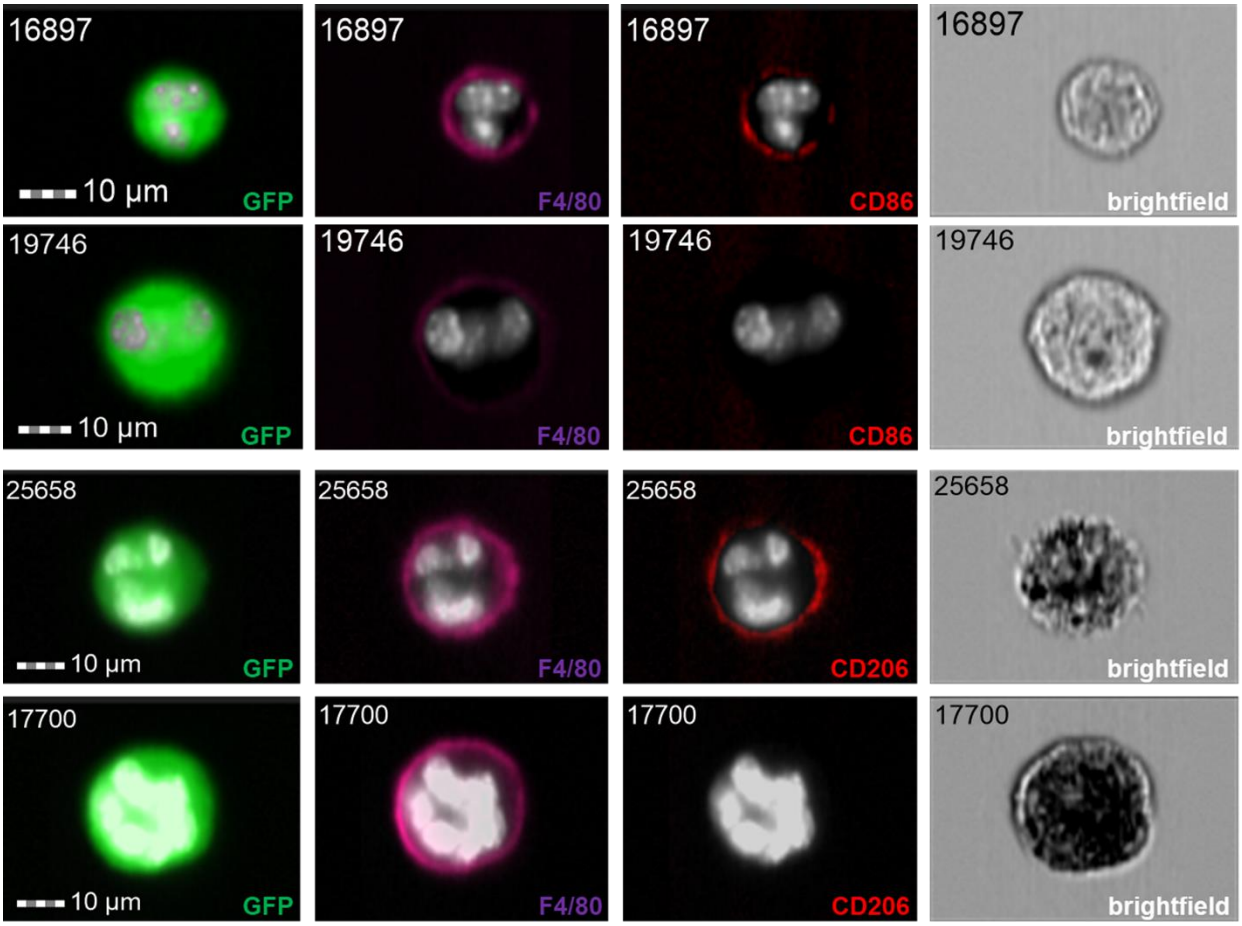
Julia Braune[1], Andreas Lindhorst[1,2], Janine Fröba[1,2], Constance Hobusch[2], Peter Kovacs[3], Matthias Blüher[3], Jens Eilers[4], Ingo Bechmann[2] and Martin Gericke[1]

Supplemental Figure 1



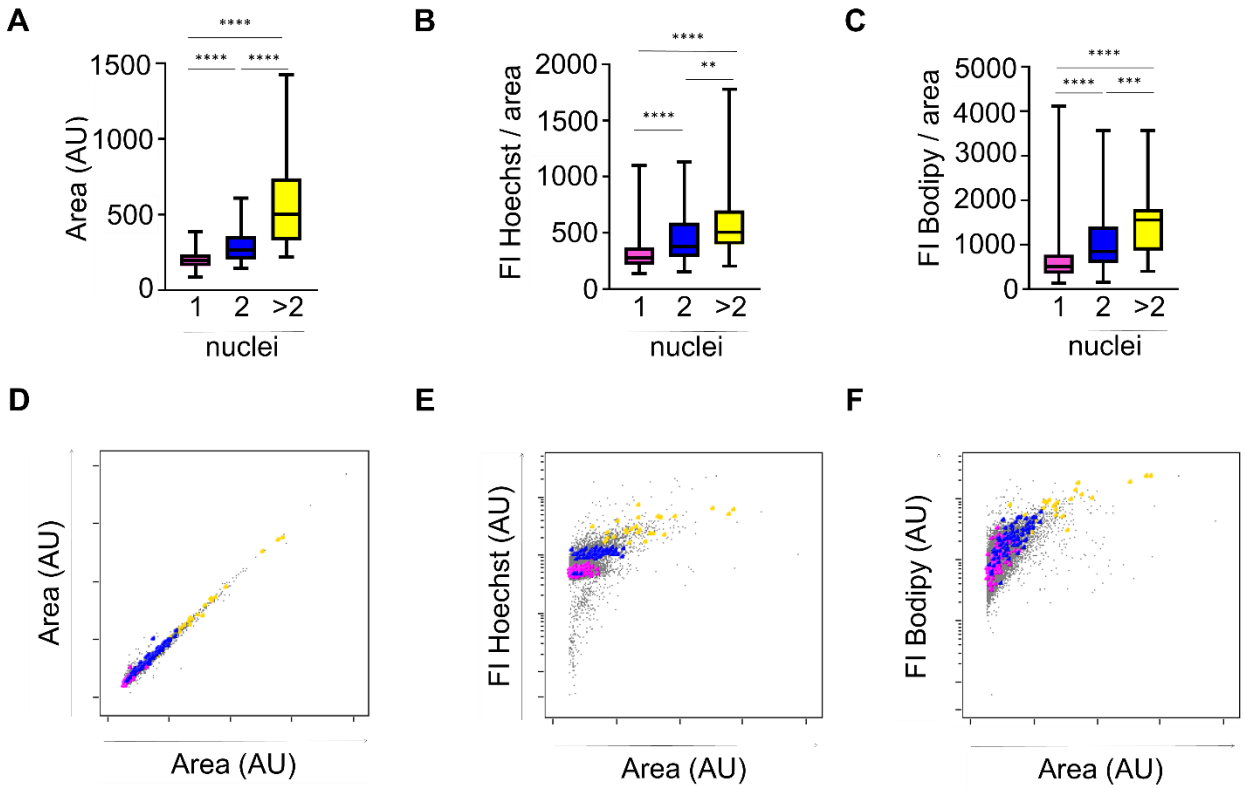
Gating strategy for flow cytometry analysis of ATMs (defined as $CD45^+$ $F4/80^+$) in obese AT explants following 48 h of cultivation. Expression of different surface markers was measured by mean fluorescence intensity.

Supplemental Figure 2



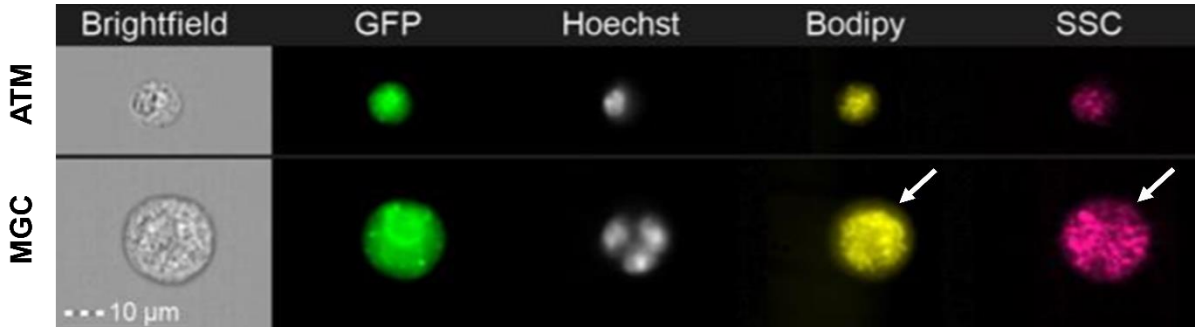
Representative images of flow cytometric imaging of CD206 and CD80 (red). MGCs were defined as GFP^+ (green), $F4/80^+$ (magenta) and polyploid (nuclei > 3, Hoechst grey) cells. Scale bar represents 10 µm.

Supplemental Figure 3



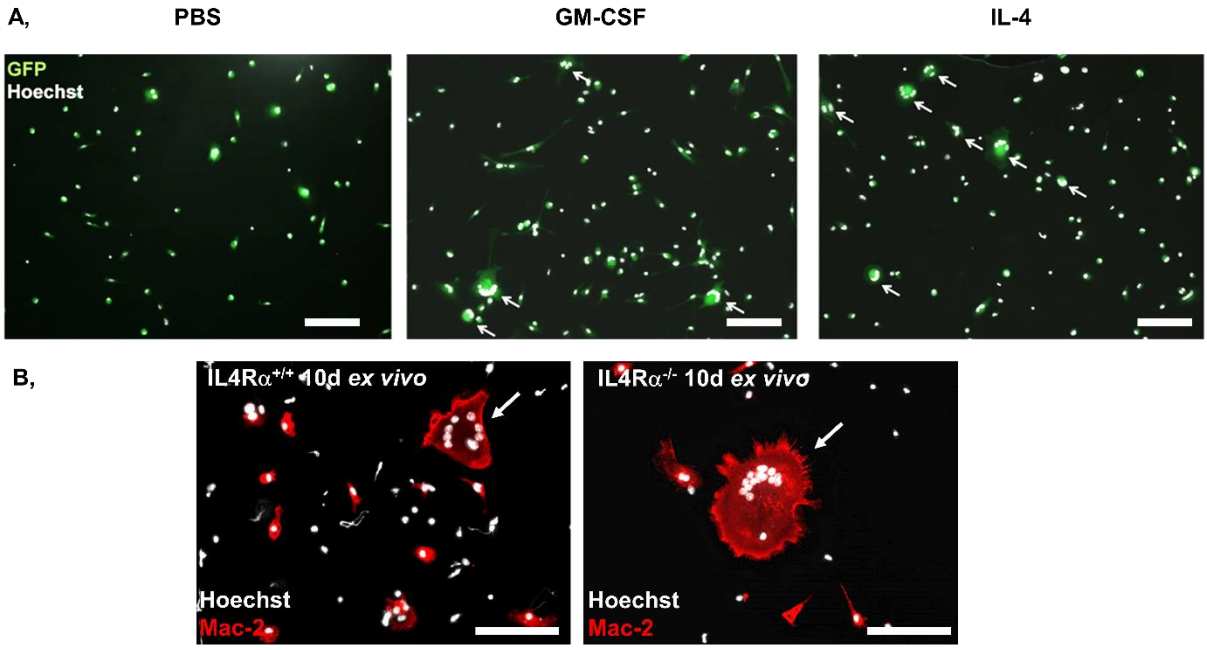
(A-C) Bar graphs represent the area (A), the relative Hoechst intensity related to the cell area (B) or the relative Bodipy intensity related to the cell area (C). (D-F) Representative flow plots of imaging cytometry. Grey dots represent all measured ATMs (defined as live $GFP^+/F4/80^+$ cells). ATMs were further sub-devided by imaging cytometry into mono-nucleated (purple), bi-nucleated (blue) and multi-nucleated ATMs. **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

Supplemental Figure 4



Representative images of flow cytometric imaging of cell lipid content visualized by the neutral lipid stain Bodipy (yellow) and granularity, measured by SSC (purple) in mono-nucleated ATMs and MGCs. MGCs were defined as GFP^+ (green), $F4/80^+$ (magenta) and polyploid (nuclei > 3, Hoechst grey) cells. Arrows indicate missing co-localization in MGCs. Scale bar represents 10 µm.

Supplemental Figure 5



IL-4 increases MGC numbers, but is dispensable for MGC formation *ex vivo*. (A) Representative images of SVF cell quantification following isolation of MacGreen mice after explant cultivation for 48 h and cytokine stimulation (50 ng/ml). (B) Representative images of SVF cell quantification following isolation of IL4Ra$^{+/+}$ and IL4Ra$^{-/-}$ mice after explant cultivation for 10 d *ex vivo* to induce MGC formation. Arrows indicate MGCs, defined as polyploidy (n>3 nuclei) macrophages. Scale bar represents 100 µm.