

Supplementary Information

Supplementary information for this article includes:

Figure S1-3

Movies S1-3

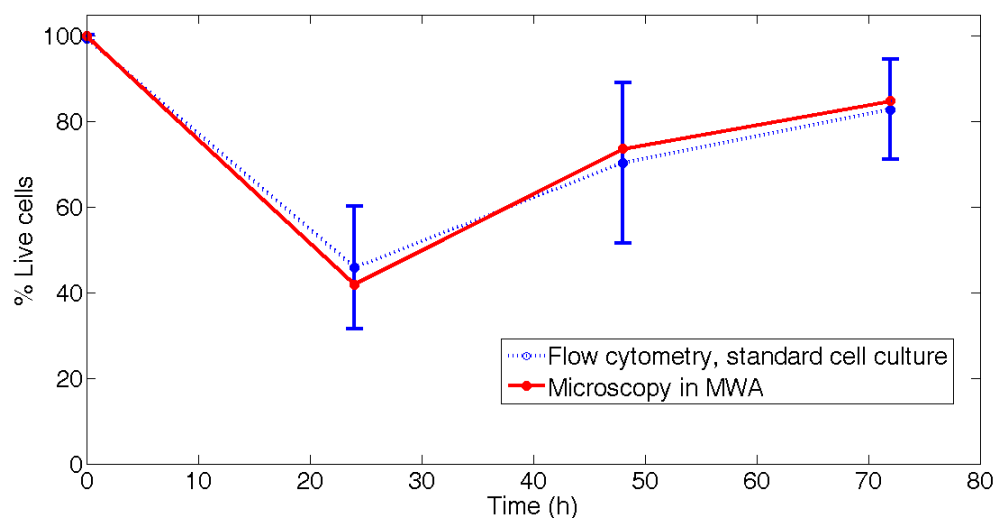


Figure S1: Comparison of cell survival in MWA and standard cell culture in a 96 well plate. CD4 T cells were cultured for 72h either in a flat bottom 96 well plate, activated with activation microbeads (blue, dashed line), or in a MWA as described in the main text (red, solid line). Percentage of live cells was evaluated by live-cell staining (LIVE/DEAD® Fixable Dead Cell Stain Kit, Invitrogen) using flow cytometry for the standard culture (2 independent experiments, 2 technical replicates each), and using PI staining for the MWA, as described in the main text.

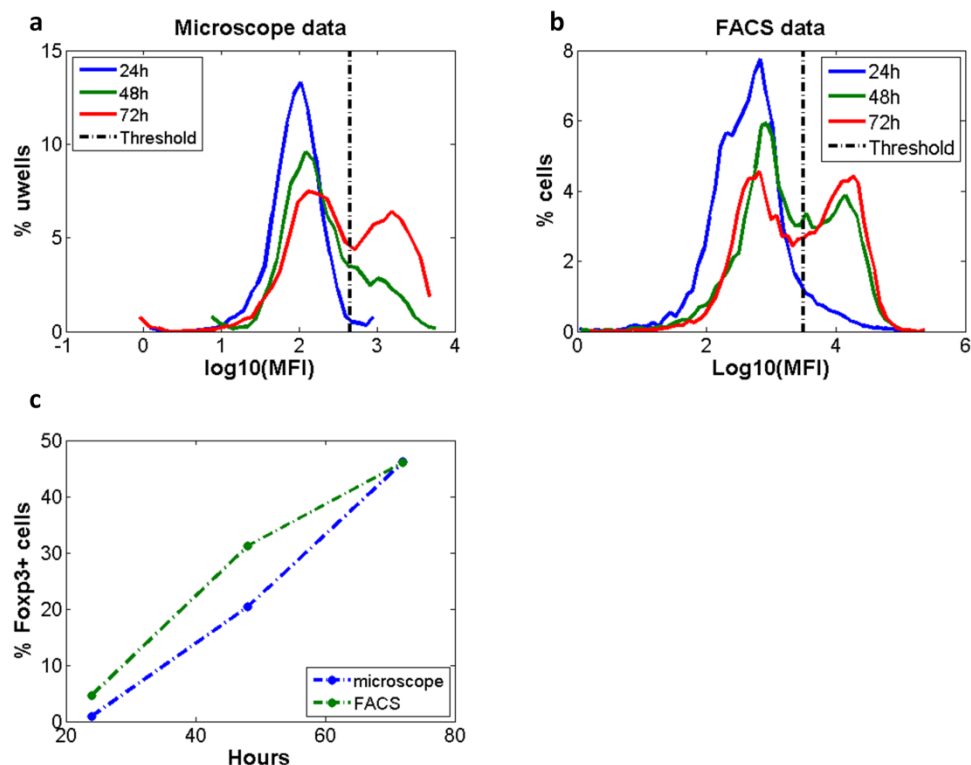


Figure S2: Comparison of Foxp3 expression in differentiating Tregs cultured in a MWA or a standard cell culture in a 96 well plate. CD4 T cells from Foxp3-GFP mice were cultured for 72h either in a MWA as described in the main text or in a flat bottom 96 well plate, activated with anti-CD3 and anti-CD28. The levels of Foxp3 expression and % of Foxp3 expressing cells were evaluated every 24h using flow cytometry for the standard culture, and tracking of GFP levels for the MWA, as described in the main text. a). Distribution of the mean fluorescent intensity (MFI) of Foxp3-GFP cells cultured in MWA. b). Distribution of the mean fluorescent intensity of Foxp3-GFP cells grown in standard culture. c). Percentage of Foxp3 expressing cells in MWA (blue line) and in standard culture (green line), using the threshold levels indicated in a and b.

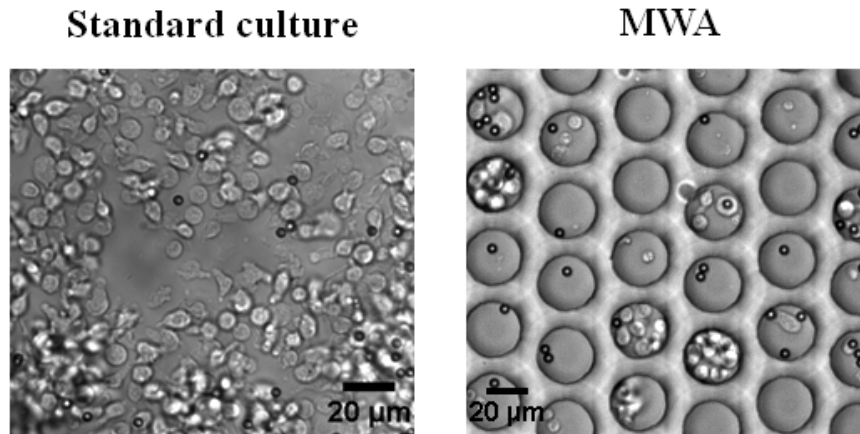


Figure S3: DIC images of T-cells in standard culture and in μ wells. Cells were stimulated using anti-CD3 anti-CD-28 coated micro-beads and cultured for 72h in a standard culture (left) or in a MWA (right). Cells that are grown in a regular culture form large clusters interfering with single cell image analysis, while cluster formation in the MWA is restricted to the μ wells.

Movie S1: A representative μ well (cropped from the full field of view), with a diameter of 25 μ m, within a MWA. The μ well initially contains one T cell and one activation bead (black). The cell divides several times during the 72 h movie (1st division: ~42h, 2nd and 3rd divisions: ~56h).

Movie S2: Another μ well from the same experiment. Here, after the first cell division (~36h), one of the daughter cells dies (at ~62h), as indicated by its staining with PI (red). The other daughter cell keeps dividing.

Movie S3: A representative view of a larger area of the MWA, containing 6 μ wells. Cells in μ wells without beads die. Cells in μ wells with beads either die or proliferate.