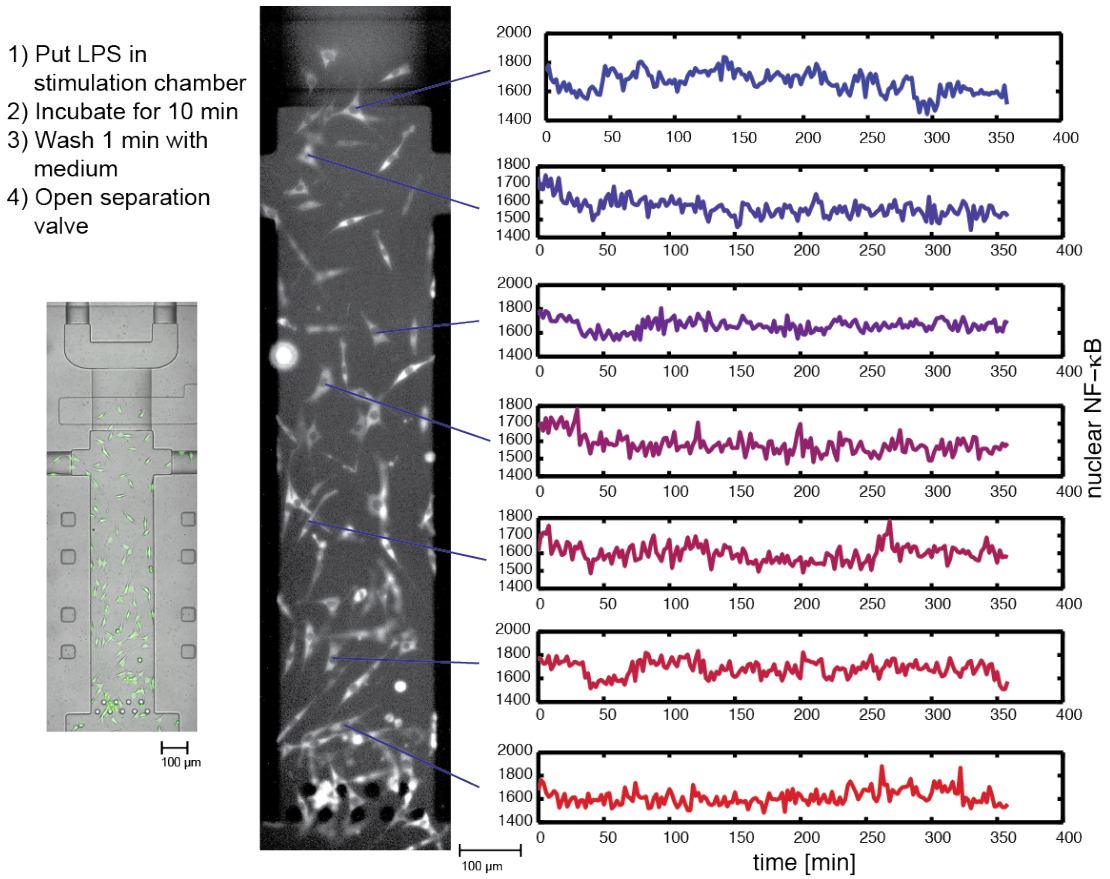
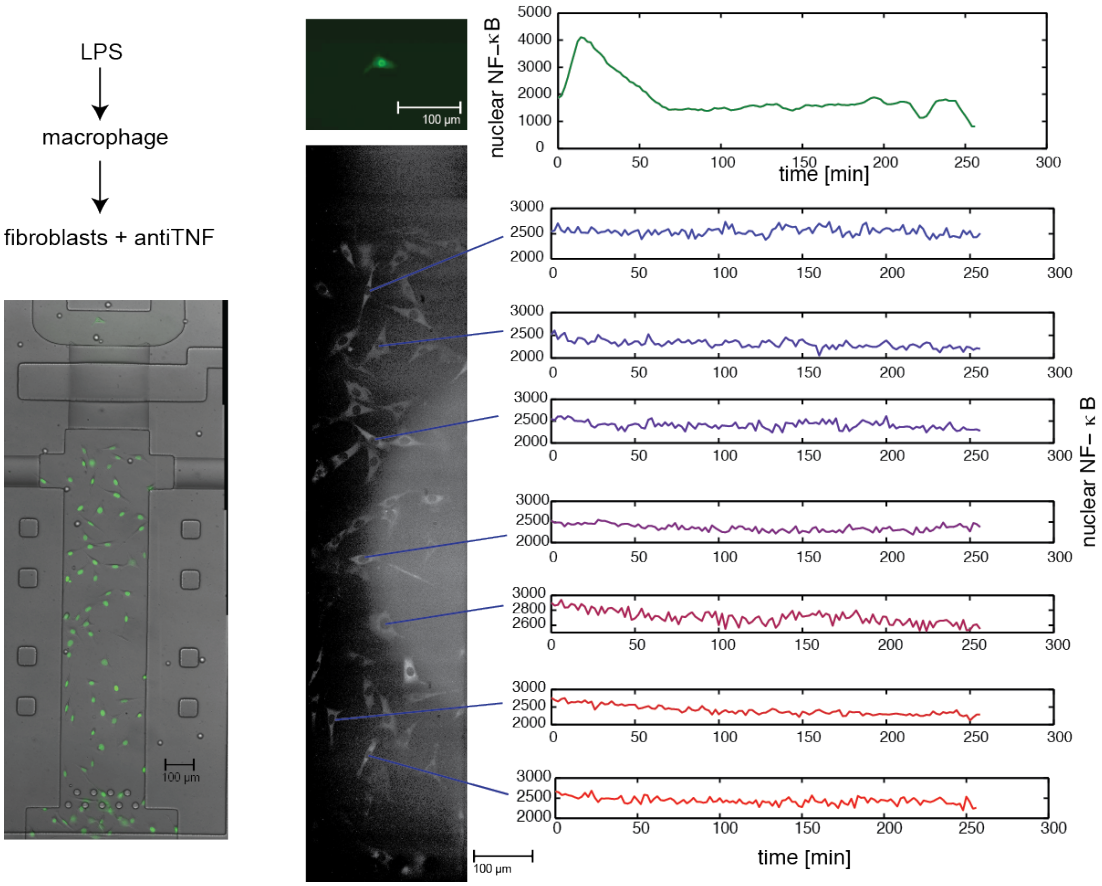
Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Material



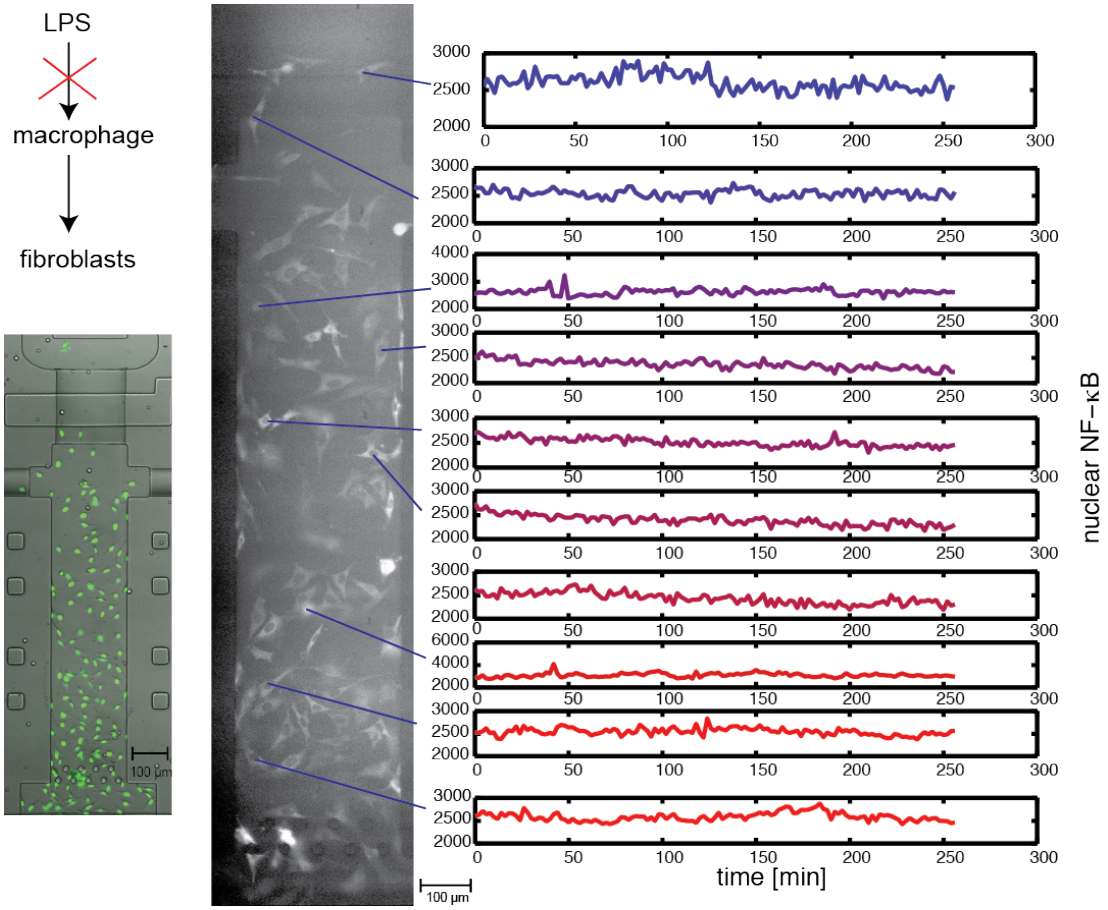
Supplementary Figure 1

LPS can be efficiently washed from the sender chamber. We filled the top stimulation chamber with LPS (c=50 ng/mL) and incubated for 10 minutes. We washed this chamber for 1 minute with fresh medium, in order to remove all LPS. We then opened the separation valve and exposed the stimulation chamber to a population of 3T3 cells. The fibroblast cells are not activated (note the lack of p65 oscillations in the fibroblast population), showing that LPS is efficiently removed from the sender chamber. The small image at the left bottom shows the bright field image of the experiment combined with the GFP channel that shows the nucleus of the cells. The larger image on the right shows the DsRed channel that shows the p65 marker, and the corresponding nuclear p65 levels in individual cells (on the right).



Supplementary Figure 2

TNF is the main signaling molecule secreted by the LPS stimulated macrophage. We cultured a single RAW macrophage (middle top) in the stimulation chamber and stimulated it as in the experiment shown in Figure 4, with two 10 minute pulses of LPS (c=50 ng/mL) with a 10 minute break in between. We washed this chamber for 1 minute with fresh medium that contains 100 µg/mL of TNF antibody, in order to remove all LPS and block secreted TNF. We then opened the separation valve and exposed the RAW cell to a population of 3T3 cells that are cultured in medium containing TNF antibodies (c=100 µg/mL). While the macrophage showed nuclear NF-κB accumulation (right-top), the fibroblast population showed no NF-κB activation (right-bottom), showing that TNF is the main secretion agent and causing the first activation of the cells upon LPS stimulation.



Supplementary Figure 3

Unstimulated macrophages do not activate the fibroblasts. We cultured two RAW macrophages in the stimulation chamber and opened the separation valve and exposed the RAW cells to a population of 3T3 cells. There was no LPS applied to the macrophages. Spatially encoded activation of NF-κB was not observed. There were few single cells showing a weak activation due to spontaneous self-activation. The trace of cell number 8 is an example of the weak activation.