



- Protease solution inlet
- Sheath fluid inlet
- Outlet port
- Chamber to be analyzed
- on-chip flow cytometry

**Supplemental figure 5.** Device operation for on-chip flow cytometry. After stimulation, fixation, and staining, cells were detached one chamber at a time by a combination of shear force and protease cleavage. A. Protease solution was flown into the inlet port (red circle) and detached cells from the chamber to be analyzed by flow cytometry (pink shade). The detached cells were then hydrodynamically focused by two sheath streams coming from the two sheath ports (yellow circles), before entering the point of detection for on-chip flow cytometry (red square). After all cells have been detached in the chamber shown in A, the remaining chambers B, C, and D are detached and analyzed in that precise order as to avoid cross-contamination of samples between chambers.