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

Supplementary Figure 1: Cell viability in droplets remains above 90% after 9 hours. Droplets containing NY-ESO-1 TCR cells alone, MART-1 TCR cells alone, NY-ESO-1$^+$ target cells alone or a mixture of NY-ESO-1 TCR cells and NY-ESO-1$^+$ target cells were collected after 3, 6 or 9-hour incubation and cells were recovered to measure the viability. For each time-point, three independent counts were done, and the mean value was used. Graph shows the % of viable cells (mean+SD) for three independent experiments.

Supplementary Figure 2: The experimental cell distribution of TCR T cells and target cells in droplets are compared to theoretical distribution. A) Labeled NY-ESO-1 TCR T cells (green) and target cells (orange) were co-encapsulated, then droplets were loaded on the iFDA chip for imaging. Picture shows cell distribution. Enlarged areas 1, 2 and 3 show 1:1, 1:2 and 1:3 cell ratios (NY-ESO-1 TCR T cell to target cell(s)) respectively. Scale bar=100 μm. B) Graph shows the theoretical distribution following Poisson statistics (black bars) and the distribution of the experimental data (grey bars) for one NY-ESO-1 TCR T cell and one target cell (1:1), one NY-ESO-1 TCR T cell and more than one target cell (1:>1), and other conditions (empty droplets, NY-ESO-1 TCR cells alone, target cells alone or >1 NY-ESO-1 TCR cell with target cells). C) Labeled NY-ESO-1 TCR T cells (green), MART-1 TCR cells (blue) and target cells (orange) were co-encapsulated, then droplets were loaded on the iFDA chip for imaging. Picture shows cell distribution. Enlarged areas 1 and 2 show 1:1 and 1:2 cell ratios (MART-1 TCR T cell to target cell) respectively, and the enlarged area 3 shows 1:1 cell ratios (NY-ESO-1 TCR T cell to target cell). Scale bar=100 μm. D) Graph shows the theoretical distribution following Poisson statistics (black bars) and the distribution of the experimental data (grey bars) for one TCR T cell and one target cell (1:1), one TCR T cell and more than one target cell (1:>1), and other conditions (empty droplets, TCR cells alone, target cells alone or >1 TCR cell with target cells).

Supplementary Video 1: Droplets are efficiently loaded and remain stationary during cleaning of remaining droplets. Droplets generated from the flow-focusing device were directed into the iFDA within two minutes. Once all of the trapping wells were filled with droplets, the iFDA chip was disconnected from the droplet generator, and extra droplets inside the iFDA chamber were removed by injecting oil for one minute. The droplet coverage rate (number of trapped droplets divided by total number of trapping wells) is above 99.9%. The capture efficiency rate (number of trapped droplets divided by total number of generated droplets) is 3%.

Supplementary Video 2: Activation kinetics is monitored on iFDA following NY-ESO-1 TCR cells co-encapsulation with NY-ESO-1$^+$ target cells. NY-ESO-1 TCR T cells were co-encapsulated with NY-ESO-1$^+$ target cells, then T cell activation was monitored over time from 0 to 9 hours through eGFP signal. Well 1: empty droplet, well 2: one target cell and one NY-ESO-1 TCR T cell showing activation, well 3: one target cell and one NY-ESO-1 TCR T cell showing no activation, and well 4: one NY-ESO-1 TCR T cell alone. Green: eGFP indicates TCR T cell activation, orange: target cell. Scale bar =50μm.

Supplementary Video 3: Droplet tracking demonstrates the accuracy of droplet sorting and dispensing. The laser-kicked droplet was tracked during the whole sorting process (iFDA, recovery chamber, pipette tip and PCR tube). Following laser-induced cavitation, the air bubble first grew in the trapping well and expelled the droplet out of the well, then the droplet was flushed into recovery chamber by injecting oil. Next, the droplet was directed to the outlet of recovery chamber by orientating the recovery chamber. Finally, the droplet was pipetted into a PCR tube for downstream molecular analysis..

Supplementary Video 4: Air bubble created by cavitation disappears within ten seconds. The bubble created by laser-induced cavitation vanished within 10 seconds due to the refreshment of cooling carrier oil, allowing efficient sorting of another target droplet. Scale bar=200μm.