Supporting Information:

A membrane-anchored aptamer sensor for probing of IFNɣ secretion by single cells

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DNA sequences

The cholesterol-linked IFNɣ aptamer probe (CLAP, 5'-/ROXN/AGGGGTTGGTTGTGTTGGGTGTTGTGTCCAACCCCT/TAO//iSp18//3CholTEG/-3') and its cDNA (cDNA2, 5'- AGG GGT TGG ACA CAA CAC CCA ACA CAA CCA ACC CCT-3') were synthesized and purified by Integrated DNA Technologies. The nine added nucleotides at the 3' end of the aptamer sequence for hairpin formation by base pairing to the 5'-end sequence are underlined.

Experiment Section

1. Fluorescence measurements

For fluorescence measurements, 5 μL aptamer probe (200 nM, in DPBS) and 5 μL cytokines of the indicated concentrations were mixed in a 0.5-mL centrifuge tube.

After incubation at 37 ^{o}C for 30 min, 90 μL DPBS was added. The mixture was transferred into a fluorescence microcuvette with an optical path length of 1.0 cm. Fluorescence emission spectra were collected from 590 to 750 nm using FluoroMax-4 spectrofluorometer at the excitation wavelength of 570 nm. The fluorescence intensity at 605 nm was used to evaluate the analysis performance of the aptamer probe.

2. Cell Isolation, Modification and Analysis

Human peripheral blood monoculear cells (PBMCs) were isolated from fresh buffy coat through ficoll gradient centrifugation. T cells were isolated from PBMCs using Human Pan T Isolation Kit (Miltenyi Biotec) and suspended in culture medium (X-VIVO medium supplemented with 2% human serum). The isolated T cells were used directly or stored in fridge for no more than 2 days.

For cell membrane engineering, T cells were washed twice with DPBS and incubated with the cholesterol-linked aptamer probe (CLAP) at room temperature (RT, ~20 ^{o}C) for certain time spans, as indicated in the text. Unless otherwise stated, a CLAP concentration of 1 μM and an incubation time of 10 min were used. Then, the cells were washed three times with DPBS to remove free probes and resuspended in fresh culture medium.

For immune stimulation assay, T cells were stimulated with 0.05 μg/mL phorbol myristate acetate (PMA) and 1 μg/mL ionomycine at 37 ^{o}C with 5% CO_2 for a certain length of time. Subsequently, the cells were collected, decorated with CLAP, and resuspended in culture medium containing PMA and ionomycine. After incubation at 37 ^{o}C with 5% CO_2 for another 2 h, the cells were washed with DPBS twice, resuspended in DBPS, and then assayed by flow cytometry (BD FACS Calibur) and/or confocal microscope (FV-1000, Olympus).

3. Fabrication of single cell-encapsulated microdroplets

PDMS microfluidic chip composed of two aqueous-phase streams and one organic-phase stream was fabricated according to our previous work.[1] To engineer a single-cell analysis platform, the CLAP-decorated cells were suspended at a concentration of 2.6×10^6 cells/mL in culture medium and delivered by one aqueous-phase stream. The immune stimuli (0.1 μg/mL PMA and 2 μg/mL ionomycine dissolved in culture medium) were delivered by the other aqueous-phase steam. Picosurf (3% w/w) in HFE 7500 was delivered by the organic-phase stream to produce water-in-oil droplets. The injection rate of both aqueous-phase streams was 200 μL/h, and that of the organic-phase stream was 900 μL/h. The cell-encapsulated droplets were collected and placed at 37 ^{o}C with 5% CO_2 and incubated for a certain length of time. For fluorescence measurements, droplets were placed on a glass slide and imaged with fluorescence microscope (Olympus CKX41).

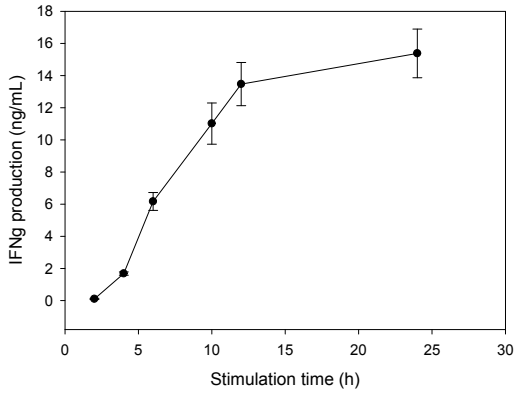

Figure S1. ELISA assay of primary T cells with stimulation of PMA/ionomycine for different time spans. Error bars represent the standard deviation of three independent experiments.

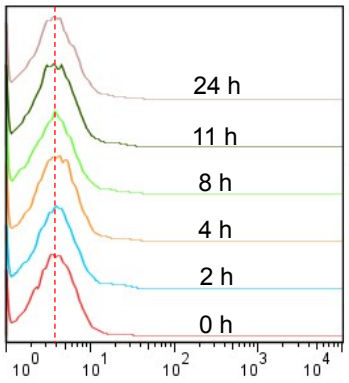

Figure S2. Flow cytometry assay of JAWS II cells with stimulation of PMA/ionomycine for different time spans, modified with CLAP and then incubated at 37 ^{o}C for another 1.5 h.

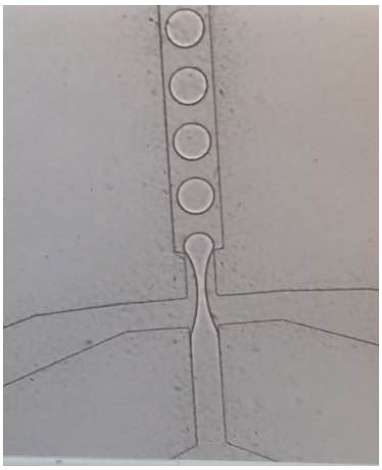

Figure S3. Fabrication of water-in-oil microdroplets with the microfluidic chip system.

1. V. Chokkalingam, J. Tel, F. Wimmers, X. Liu, S. Semenov, J. Thiele, C. G. Figdor and W. T. Huck, *Lab on a chip*, 2013, **13**, 4740-4744.