Electronic Supplementary Material (ESI) for Analytical Methods.

10th August 2020 Note: This ESI file replaces that originally published on 26th May 2020 which contained errors in Fig. S5 and S7.

Supporting Information

Sensitive detection of single-cell secreted lactic acid for glycolytic inhibitor screening with a microdroplet biosensor

Xuyue Chen[1†], Rui Shen[1†], Sidi Liu[1†], Xiang Xiao[1], Jun Yan[1], Yiqiu Zhang[1], Zhongyun Jiang[1], Baoqing Nie[2], Jian Liu[1*]

[1]Institute of Functional Nano and Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Soochow University, Suzhou, Jiangsu Province, 215123, China

[2]School of Electronic and Information Engineering, Soochow University, Suzhou, Jiangsu Province, 215006, China

† These authors contributed equally to this manuscript.

Corresponding author: Prof. Jian Liu, jliu@suda.edu.cn

iMINTS Lab, http://web.suda.edu.cn/jliu/index.html

Abstract

Lactic acid (LA) plays an important role in the tumor metabolism and malignant progression of various cancer. Herein, we have developed an one-step, free-wash microfluidic approach with droplet biosensors for sensitive detection of LA secreted by a single tumor cell. Our assay integrates an enzyme-assisted chemical conversion of LA in the small volume (4.2 nL) droplets for fluorescent signal readout. We have achieved a limit of detection (1.02 μM) with the microdroplet assay, more sensitive than the commercial ELISA kit by nearly two orders of magnitude. A good specificity has been demonstrated for this assay by testing various ions and biomolecules from the culture medium. This droplet assay allows us to acquire the profiles of lactic acid secretion of tumor cells under the influence of glycolytic inhibitors at the single-cell level. It offers a useful research tool to study the cell-to-cell differences of LA secretion and glycolytic inhibitor screening for cancer research.

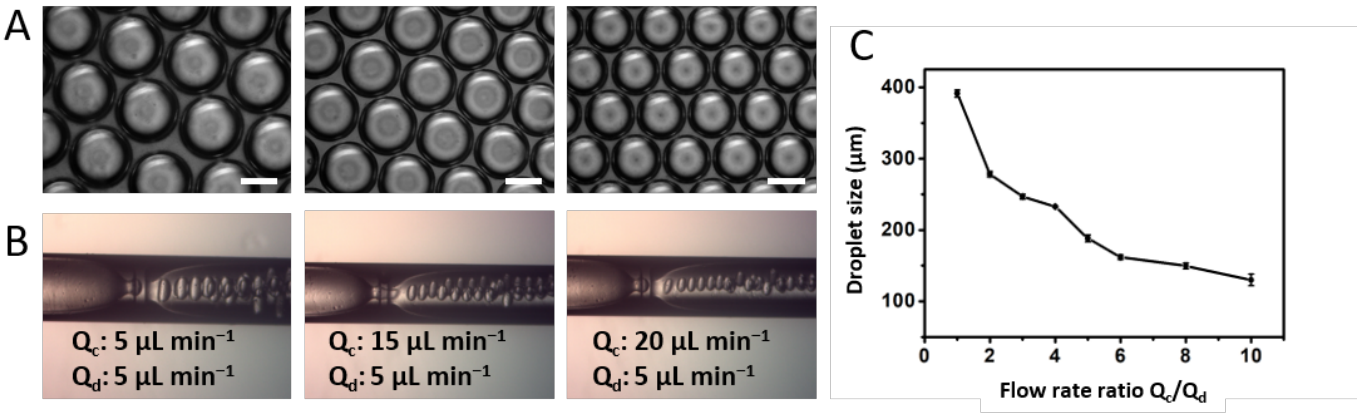

Figure S1. Generation of diameter-tunable microdroplets by changing the flow rate ratios (Q_c / Q_d). (A) Optical micrographs of the microdroplets in the diameters of 400 µm, 300 µm, and 200 µm. Flow rates of Q_c: 5, 15, 20 µL min^{-1}, Q_d: 5 µL min^{-1}. Scale bar:200 µm. (B) Video clip snapshots during experiments of droplet generation. (C) Calibration curve of the droplets diameter at the different flow rate ratios (Q_c / Q_d). Error bar: standard deviation from different images (n=5).

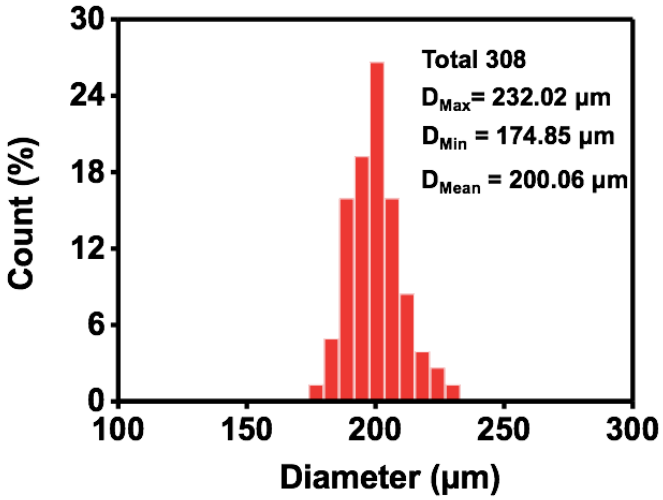

Figure S2. Size distribution of the droplets from the 7 different batches at the fixed flow rates (Q_d: 5 µL min^{-1}; Q_c: 20 µL min^{-1}). The number of droplets in total: n=308.

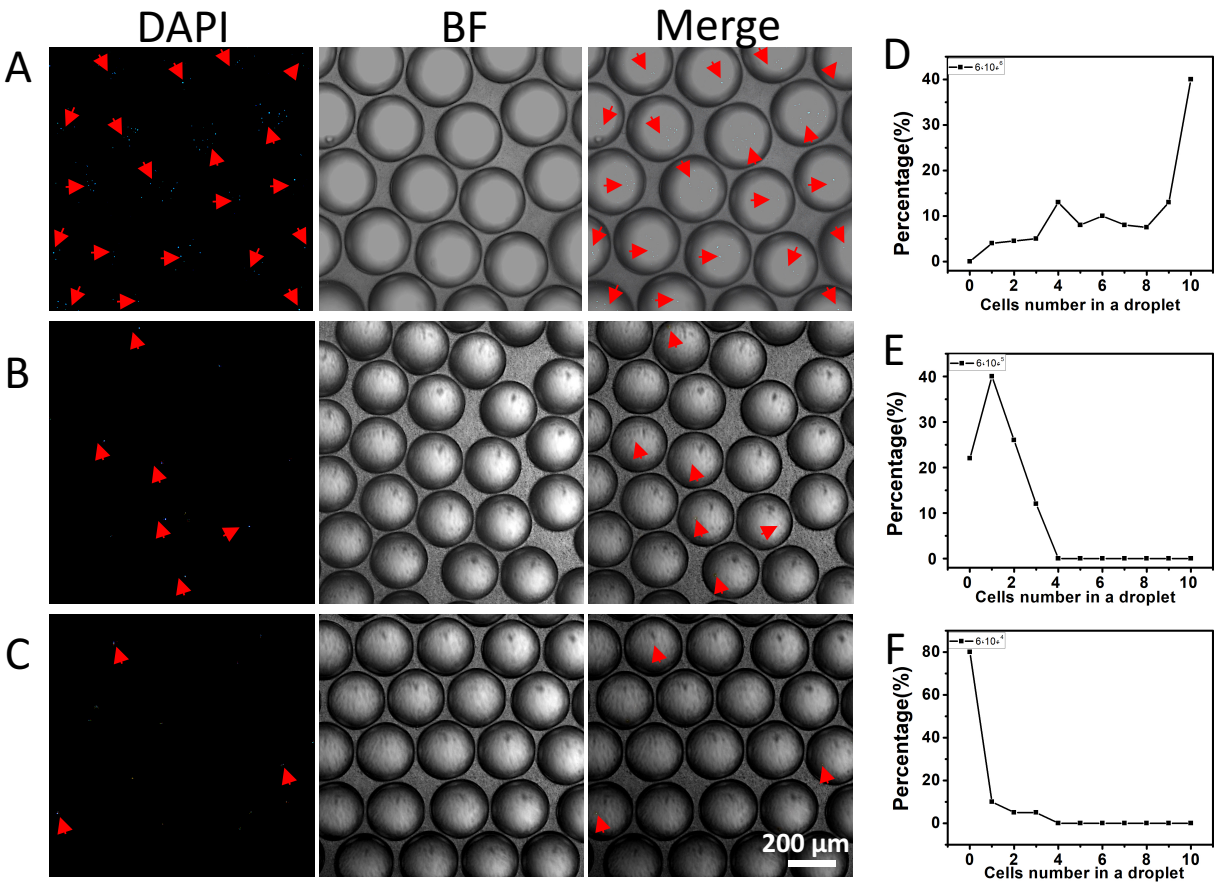

Figure S3. Cell density titration experiments for optimized single-cell encapsulation with droplets. (A-C) The images of droplets in the DAPI channel, bright field, and merged. A549 cell concentrations: 6×10^6 cells mL^{-1}, 6×10^5 cells mL^{-1}, and 6×10^4 cells mL^{-1}. Scale bar: 200 µm. The red arrows as a visual guide for cell location inside the droplets. (D-F) Percentage of the possibility versus cell number in each microdroplets.

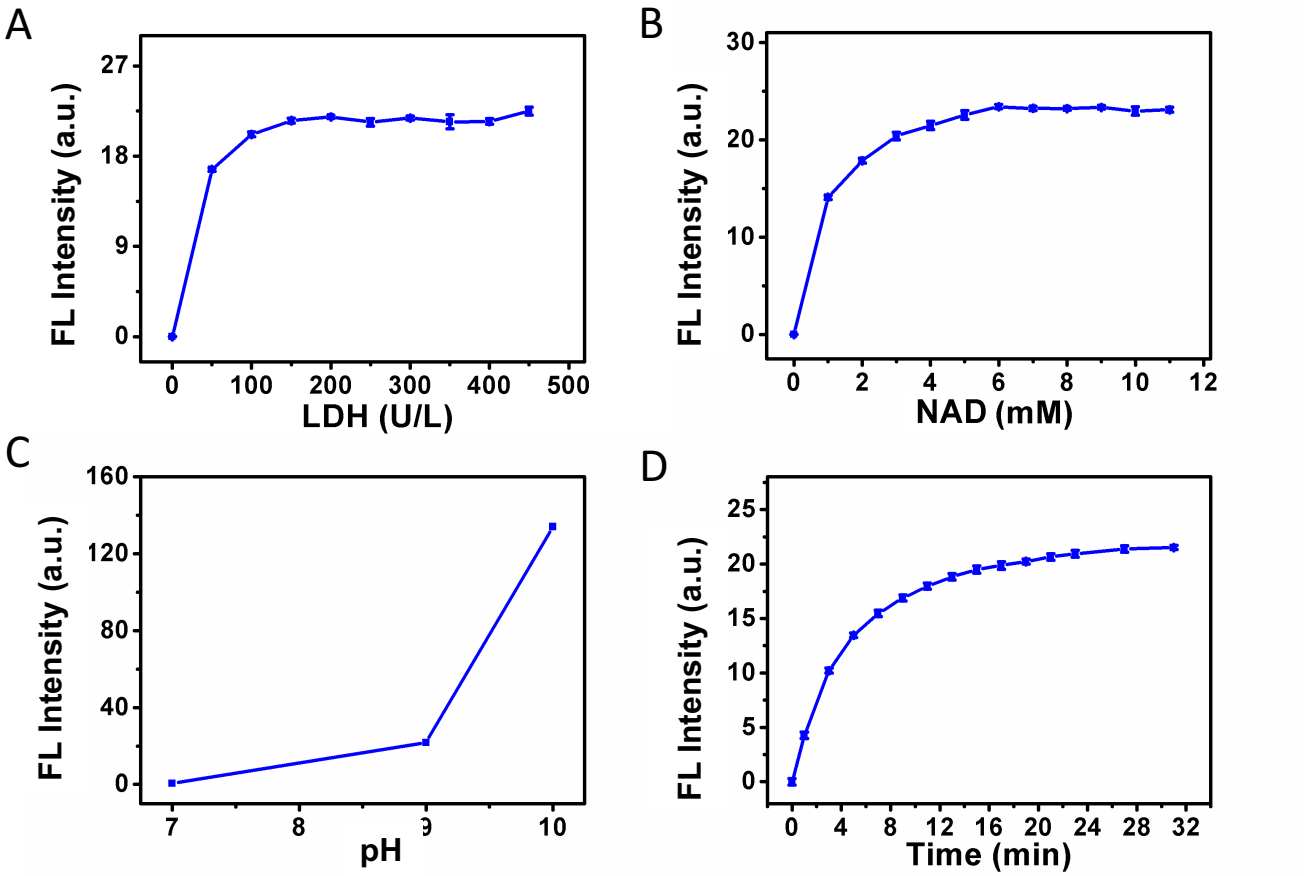

Figure S4. Optimization of the fluorescence assay for the detection of lactic acid. (A) Titration of lactate dehydrogenase (LDH) concentrations. LA: 25 mM, NAD: 6 mM. (B) Titration of nicotinamide adenine dinucleotide concentrations. LA: 25 mM, LDH: 200 U L^{-1}. (C) Titration of the buffer pH values. (D) Tests of different incubation time of the reagent mixture.

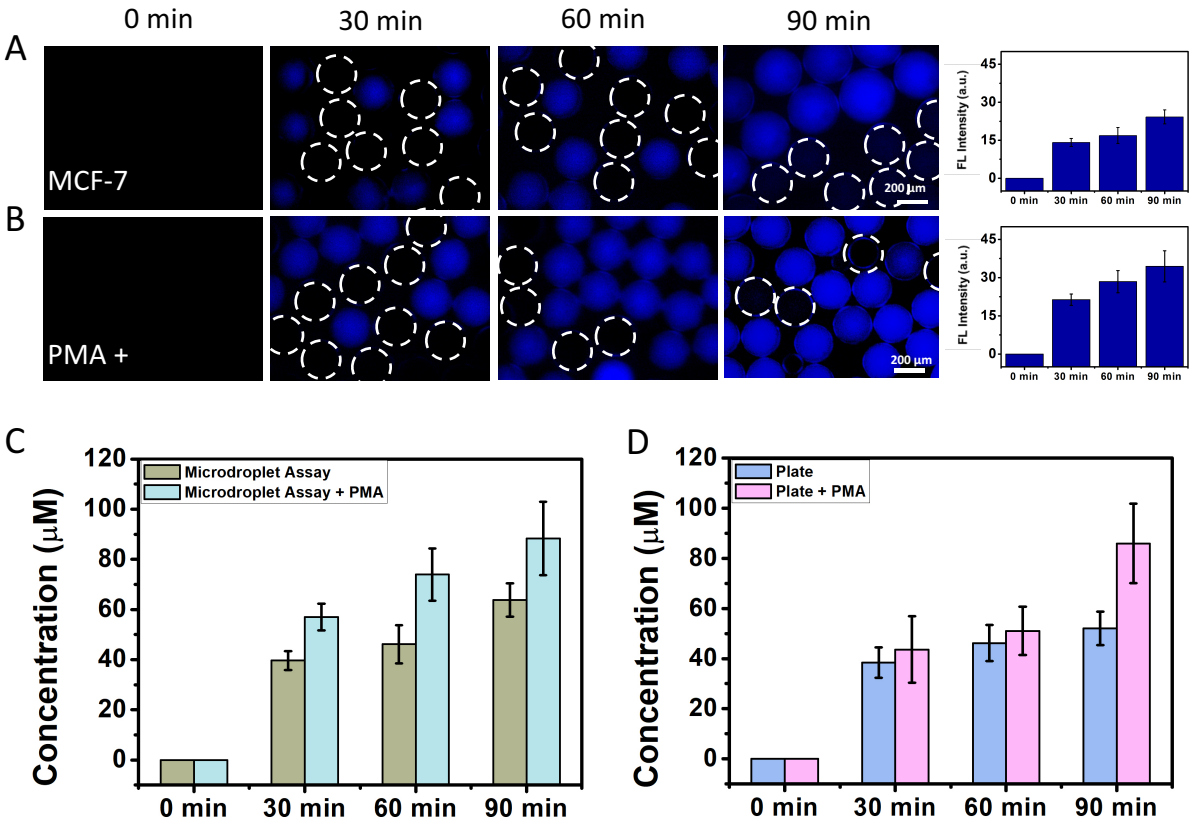

Figure S5. Fluorescent measurement of droplets encapsulating single MCF-7 cells at the time points of 0, 30, 60 and 90 min. (A-B) Fluorescent images and intensity analysis of MCF-7 cells without or with PMA stimulation. Ex/Em : 360 nm/490 nm. The white circles: the empty microdroplets. Scale bar: 200 μm. Error bar: standard deviation from different cubes (n=3). (C) Lactic acid secretion of a single MCF-7 cell using the droplet assay. Error bar: standard deviation from different cubes (n=3). (D) The averaged lactic acid concentrations secreted by MCF-7 divided by the cell numbers in the microplate (2.4×10^5 cells/well) using the commercial kit. Error bar: standard deviation from different experiments (n=3).

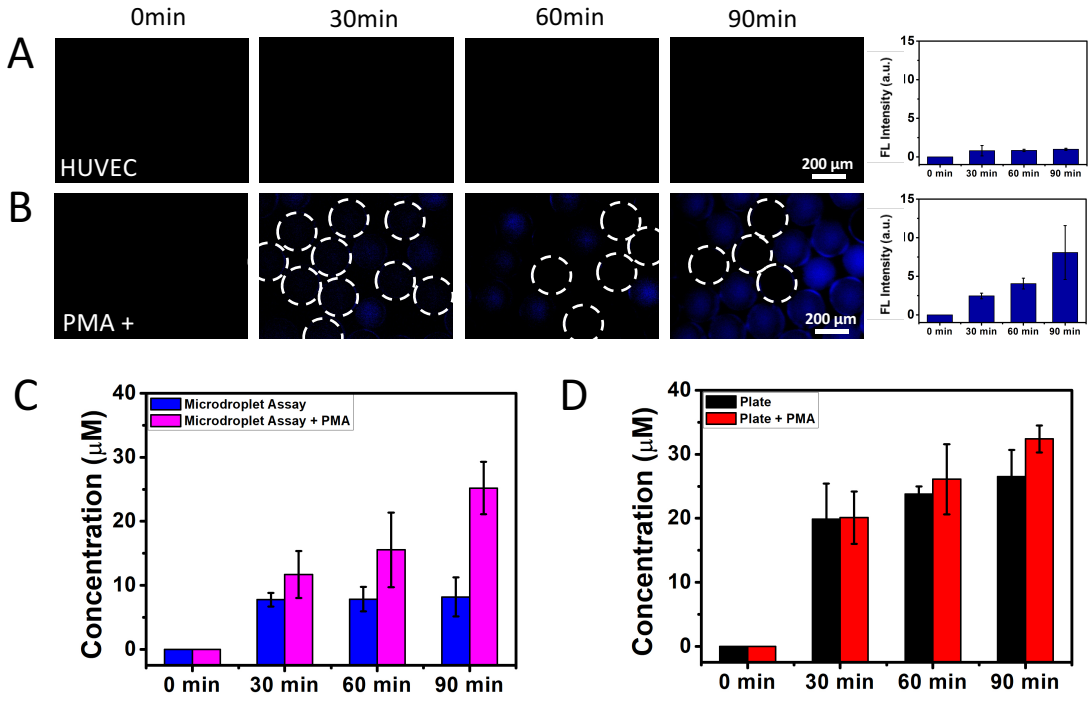

Figure S6. Fluorescent measurement of droplets encapsulating single HUVEC cells at the time points of 0, 30, 60 and 90 min. (A-B) Fluorescent images and intensity analysis of HUVEC cells without or with PMA stimulation. Ex/Em : 360 nm/490 nm. The white circles: the empty microdroplets. Scale bar: 200 μm. Error bar: standard deviation from different cubes (n=3). (C) Lactic acid secretion of a single HUVEC cell using the droplet assay. Error bar: standard deviation from different cubes (n=3). (D) The averaged lactic acid concentrations secreted by HUVEC divided by the cell numbers in the microplate (2.4×10^5 cells/well) using the commercial kit. Error bar: standard deviation from different experiments (n=3).

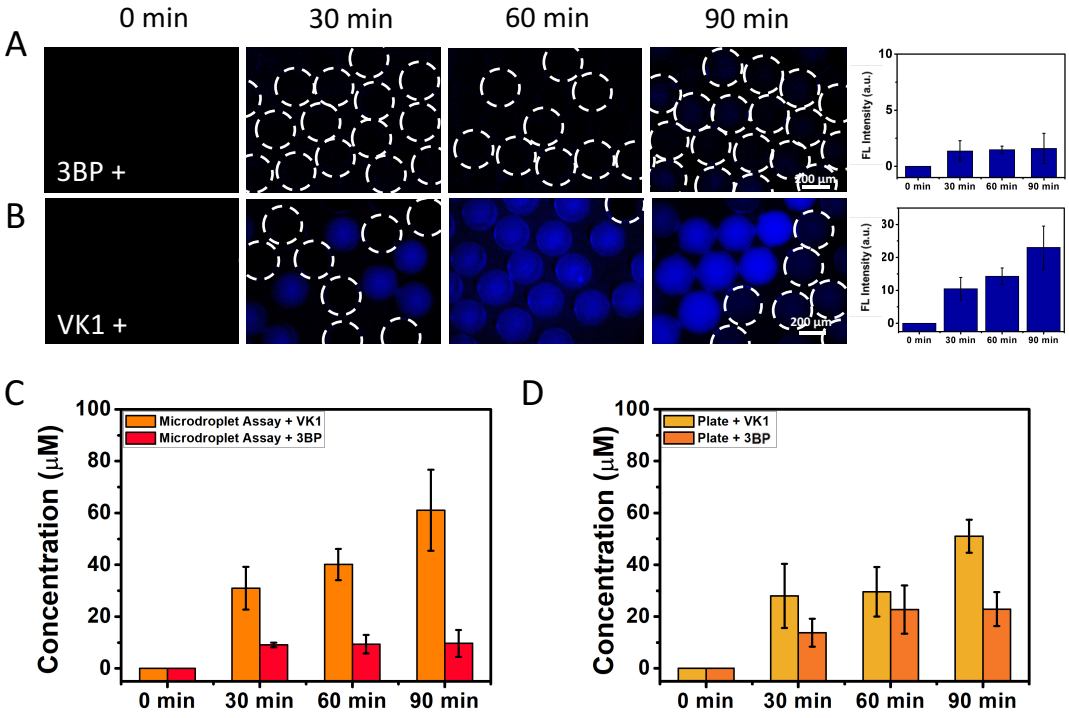

Figure S7. Fluorescent measurement of droplets encapsulating single MCF-7 cells at the different time points for glycolytic inhibitor screening. (A-B) Fluorescent images and intensity analysis of MCF-7 cells with incubation of 3BP and VK_1. Ex/Em : 360 nm/490 nm. The white circles: the microdroplets with fluorescence below the threshold, either cellular LA scretion inhibited or no cell encapsulation. Scale bar: 200 μm. Error bar: standard deviation from different cubes (n=3). (C) Lactic acid secretion of a single MCF-7 cell inhibitored by 3BP and VK_1 using the droplet assay. Error bar: standard deviation from different cubes (n=3). (D) The averaged lactic acid concentrations secreted by MCF-7 divided by the cell numbers in the microplate (2.4×10^5 cells/well) using the commercial kit, under the inhibition of inhibitored by 3BP and VK_1. Error bar: standard deviation from different experiments (n=3).

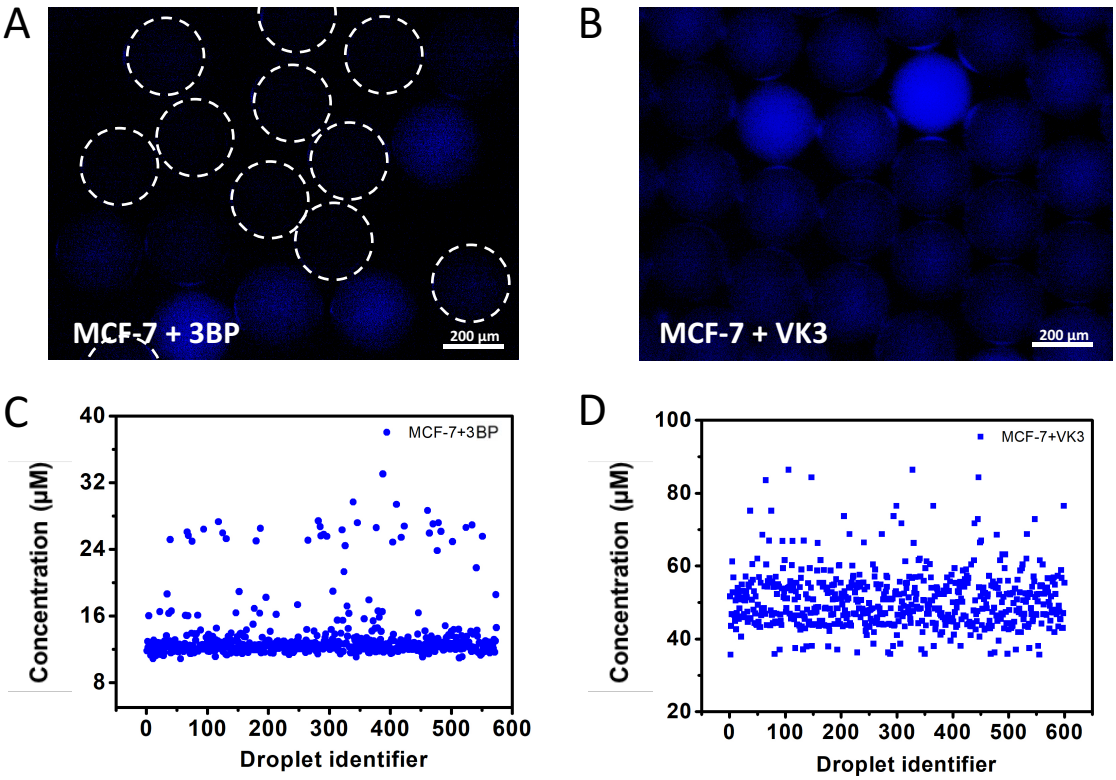

Figure S8. Lactic acid secretion of MCF-7 inhibited by 3BP or VK_3 using the droplet assay, suggesting cell-to-cell difference in glycolytic processing. (A-B) Fluorescent images of MCF-7 cell-encapsulated droplets with incubation of 3BP or VK_3. Ex/Em: 360 nm/490 nm. The white circles: the microdroplets with fluorescence below the threshold, either cellular LA scretion inhibited or no cell encapsulation. Scale bar: 200 µm. (C-D) Scatter plots of single-cell secreted lactic acid concentration in the individual droplets.