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

Supporting information for

High-throughput Probing Macrophage-bacteria Interactions at the Single Cell Level with Microdroplets

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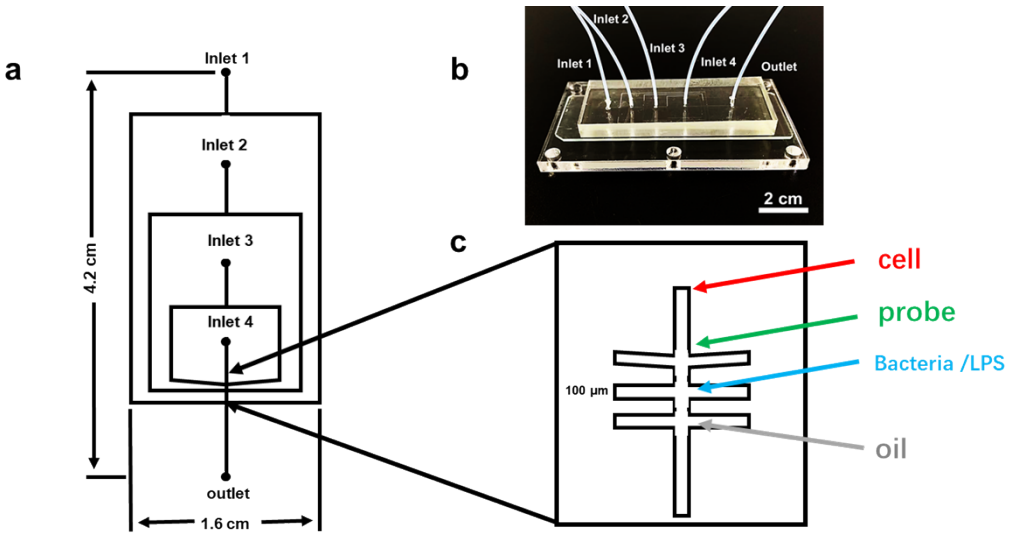

Figure S1. Specification of the microfluidic device for droplet generation. (a) Structure diagram of the microfluidic device. Length: 4.2 cm; width: 1.6 cm. (b) Photo of the microfluidic device. Scale bar: 2 cm. (c) Zoomed-in view of the cross channels for droplet generation. Feature dimension: channel width: 100 μ m; channel height: 50 μ m. The arrows in the different colors indicate the sequence of loading samples or reagents.

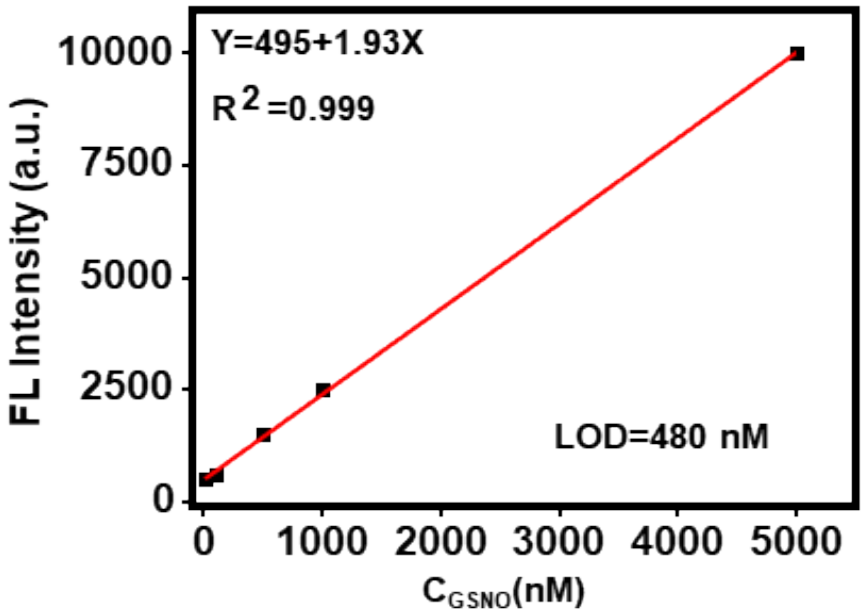

Figure S2. The standard curve of NO measured in the format of 96-well microplate. GSNO as the donor of NO; DAF-2 as the fluorescent probe. Limit of detection: 480 nM.

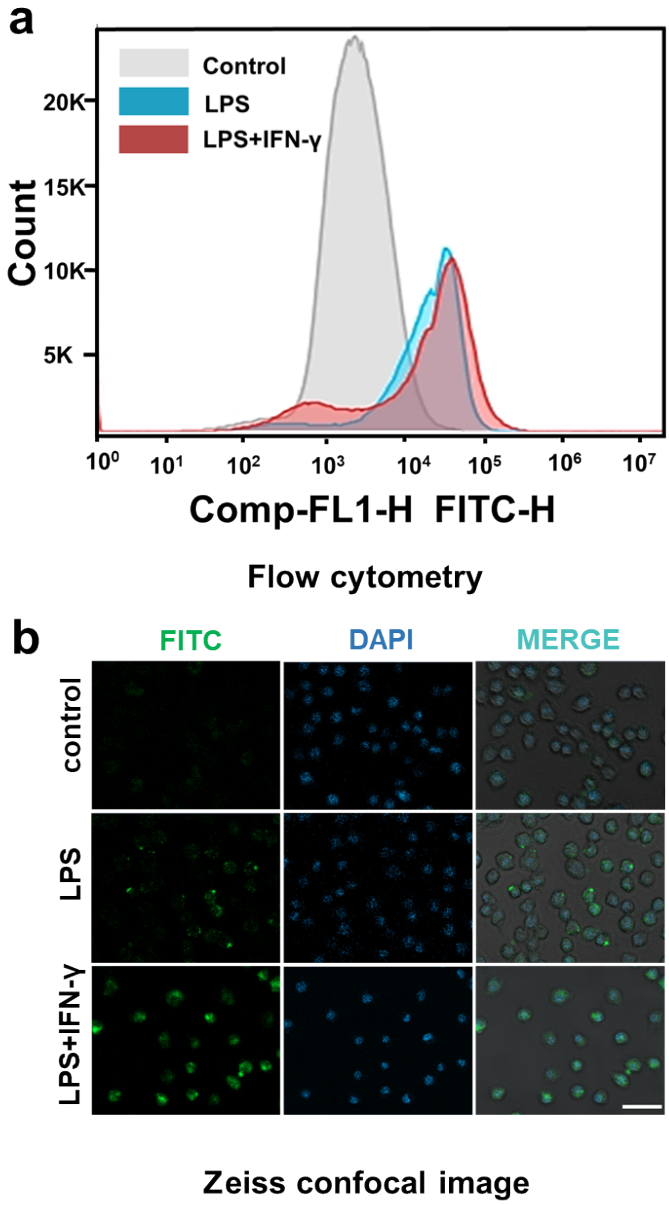

Figure S3. Validation of macrophage polarization with the probe of DAF-FM DA. (a) Flow cytometry data of the macrophage polarization by different stimuli, including PBS control, LPS, and LPS+IFN-γ. Macrophages: RAW264.7 cells. LPS concentration: 250 ng/mL; IFN-γ concentration: 100 ng/mL. (b) Fluorescent images of the macrophage polarization under the identical condition as above.

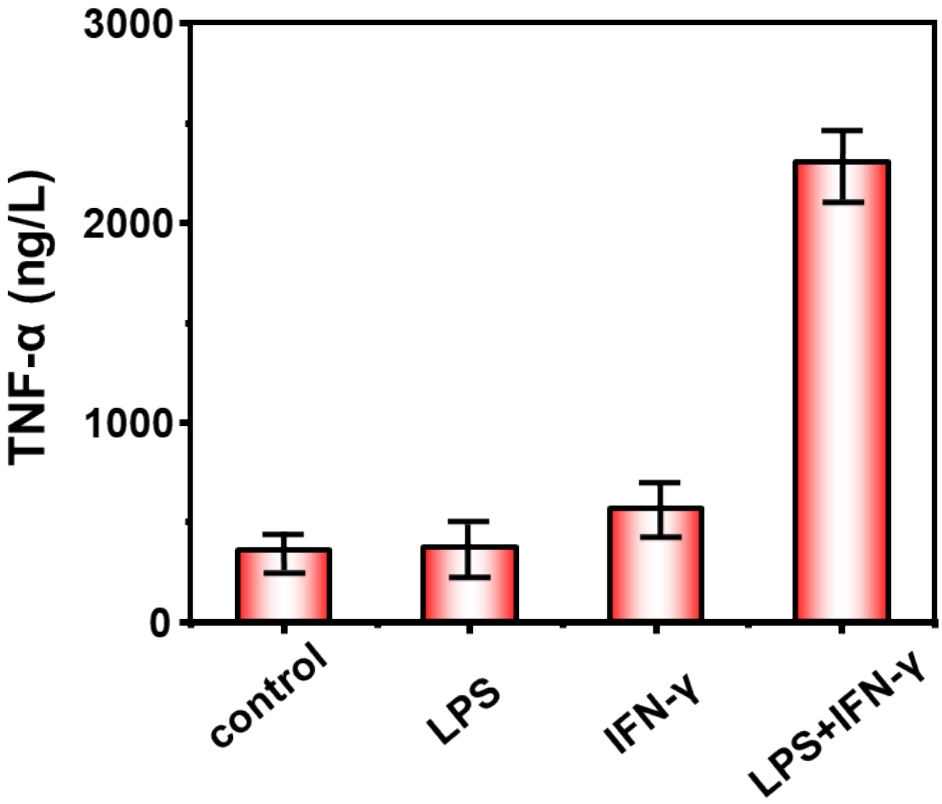

Figure S4. ELISA measurements of TNF-α secretion by the macrophages after different stimulations, including PBS control, LPS, IFN-γ, and LPS+IFN-γ. Macrophages: RAW264.7 cells. LPS concentration: 250 ng/mL; IFN-γ concentration: 100 ng/mL. Error bar: standard deviation (n=3).

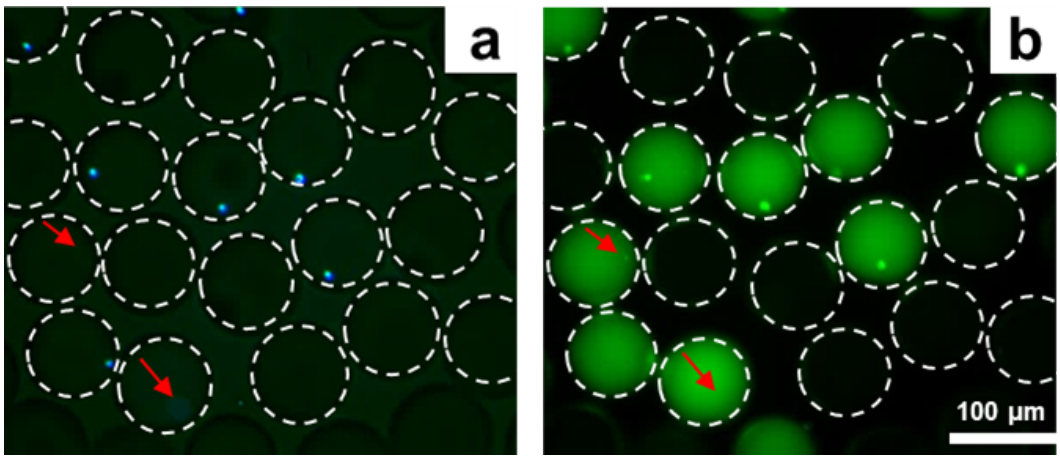

Figure S5. Detection of extracellular NO secretion by a single macrophage encapsulated in the droplet. (a) Fluorescent image at the Hoechst channel. (b) Merged image of both the Hoechst channel and the DAF-2 channel. Macrophages (RAW264.7 cells) were pre-stained by the live-cell Hoechst dye, followed the polarization step with LPS+IFN-γ. LPS concentration: 250 ng/mL; IFN-γ concentration: 100 ng/mL. White dashed circles as a visual guide for the droplets. Scale bar. 100 μm. Red arrows indicated the potential locations of the macrophage nuclei (out-of-focus during image acquisition) inside the droplets.

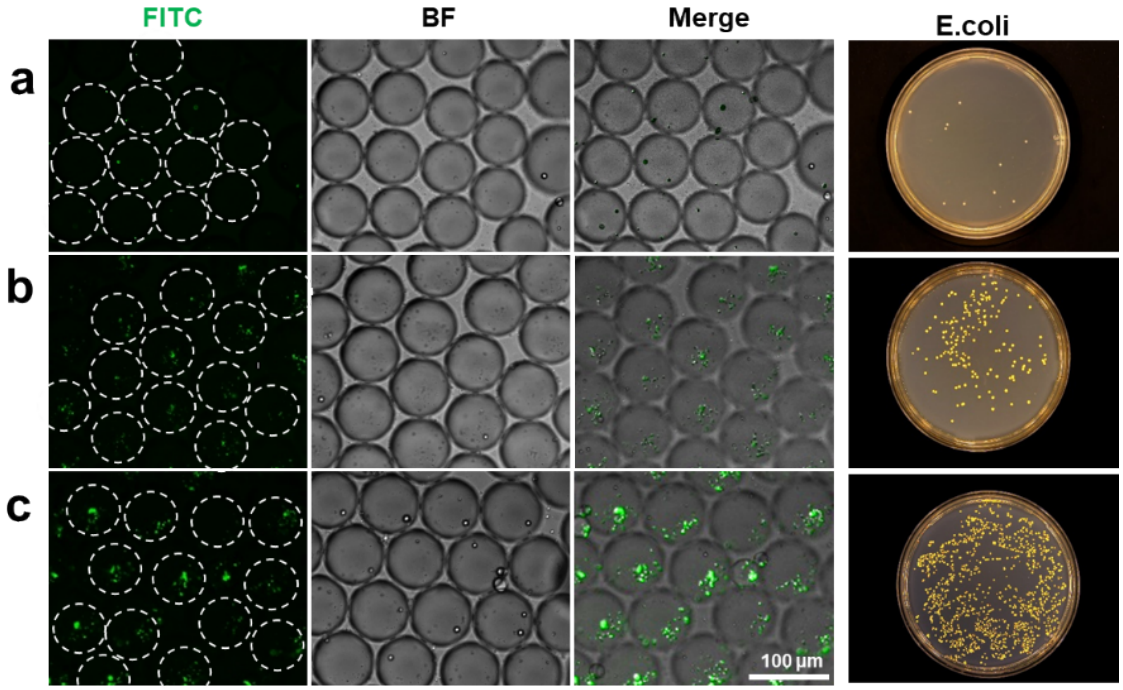

Figure S6. Encapsulation of *E. coli* by the droplets and bacteria counting. (a-c) *E. coli* loading concentrations: 1.0×10^7 CFU/mL, 1.0×10^8 CFU/mL, and 1.0×10^9 CFU/mL respectively. The number of *E. coli* in each droplet on the average: (a) 1-2 *E. coli.* per droplet; (b) 15-20 *E. coli* per droplet; (c) 150-200 *E. coli* per droplet.