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

Supplementary Information

Pressure drop and design criteria for a microfluidic SDA

The simplified Darcy-Weisbach equation used for determining the pressure drop in a hydrodynamic trap is as follows:

$$\Delta P = \frac{C(\alpha)}{32} \cdot \frac{\mu L Q (2W + 2H)^2}{A^3} \qquad \text{(Eq.1)}$$

where $C(\alpha)$ denotes a constant that is a function of α (aspect ratio of channel cross-section), L is the length of the channel, Q is the volumetric flow rate, W and H is the channel width and height, respectively, and μ is the fluid viscosity.

Because the pressure drop of path 1 to the array chamber and path 2 to the bypass channel is the same, Eq. 1 is applied separately for path 1 and path 2. Therefore, the ratio of volumetric flow rate between path 1 and path 2 can be expressed as follows.

$$\frac{Q_1}{Q_2} = \left(\frac{C_2(\alpha_2)}{C_1(\alpha_1)}\right) \bullet \left(\frac{L_2}{L_1}\right) \bullet \left(\frac{W_2 + H}{W_1 + H}\right)^2 \bullet \left(\frac{W_1}{W_2}\right)^3 > 1 \qquad \text{(Eq.2)}$$

Note that this final expression can apply for all velocities in the laminar flow regime. Supplementary Table 1 lists the dimensions of the microfluidic SDA that apply to our results.

Supplementary Table 1. Volumetric flow ratios for microfluidic SDA geometric dimensions

Actuation of trap valve	Width of trap cavity channel (W_1)	Length of trap cavity channel (L_1)	Width of bypass channel (W_2)	Length of bypass channel (L_2)	Height (H)	Q_1/Q_2
No	60	70	40	830	20	20.5
Yes	20	80	40	830	20	3.19

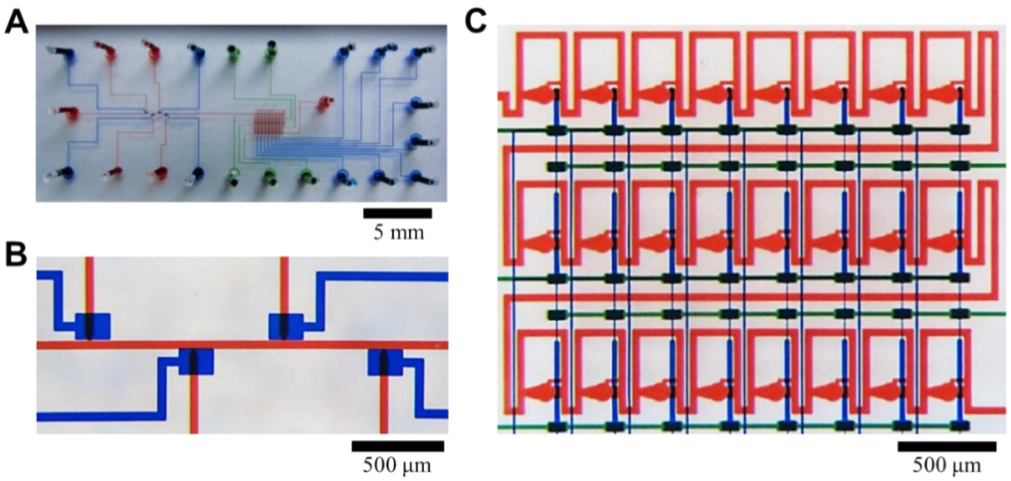

Fig. S1. Double-layer microvalve integrated microfluidic device. (A) Photograph of microfluidic device. Red, blue, and green dyes indicate the fluidic, control, and block layers. (B) Optical images of a droplet generation part and (C) SDA part containing 24 droplets in a microfluidic device

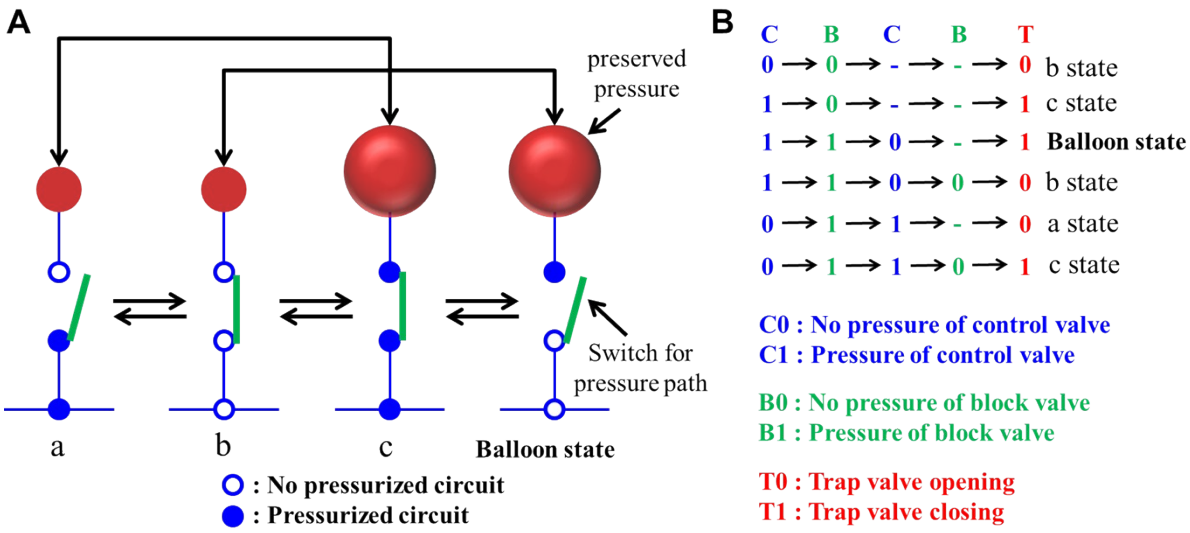

Figure S2. The principle of the "balloon" valve using double-layered microvalve system. (A) A schematic diagram for the concept of the balloon state by operation of the intermediate switch. The main pressure circuit line (blue), intermediate switch (green), and balloon (red) correspond with the control valve, block valve, and trap valve, respectively. (B) Final state of the trap valve after sequential operation of the control valve and block valve.

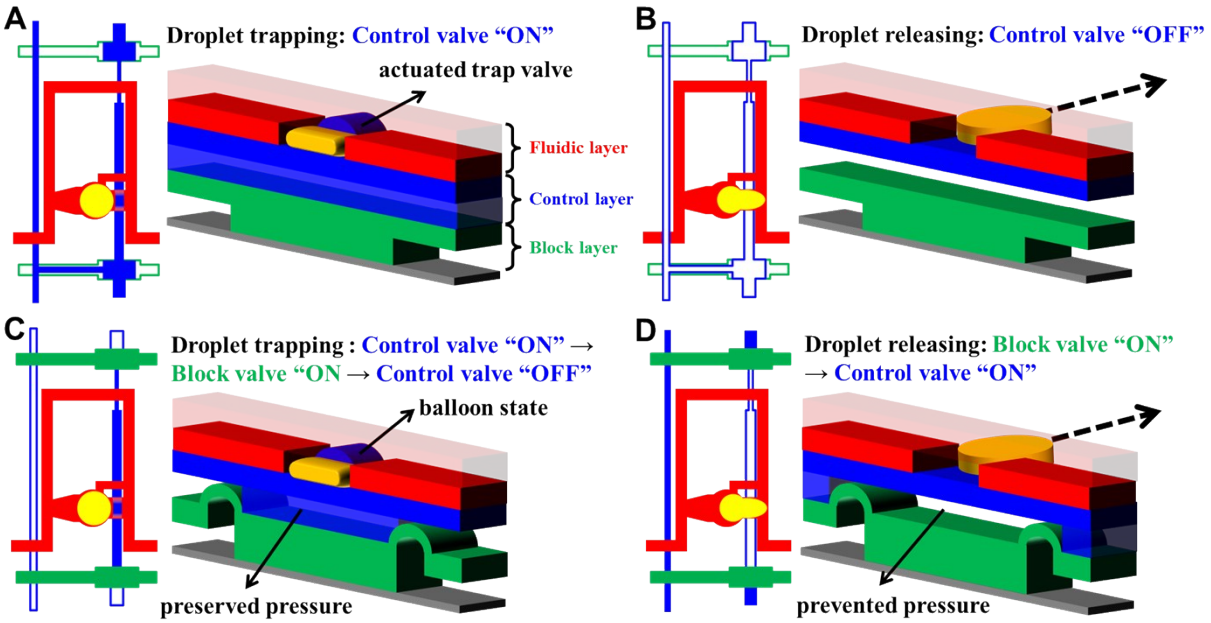

Fig. S3 Schematic diagram for droplet releasing and trapping as a sequential operation of the control and block valves. The trap valve is actuated by expanding the PDMS membrane during pressure of the on-state of the control layer, which traps a droplet in the hydrodynamic trap. The Pressure on- or off-state of the control valve determines the actuation or non-actuation state of the trap valve. (A) Actuation of the trap valve results in the on-state of the control valve, and a droplet is trapped. (B) Non-actuation of the trap valve results in the off-state of the control valve, and a droplet is released or passed. (C) For sequential operation (Control valve on → Block valve on → Control valve off), the actuated trap valve is maintained by preserving the applied pressure (balloon state of trap valve), despite the off-state of the control valve, and a droplet is trapped. D) For sequential operation (Block valve on → Control valve on), the non-actuated trap valve is maintained by preventing the pressure transmission, despite the on-state of the control valve, and a droplet is passed to the hydrodynamic trap.

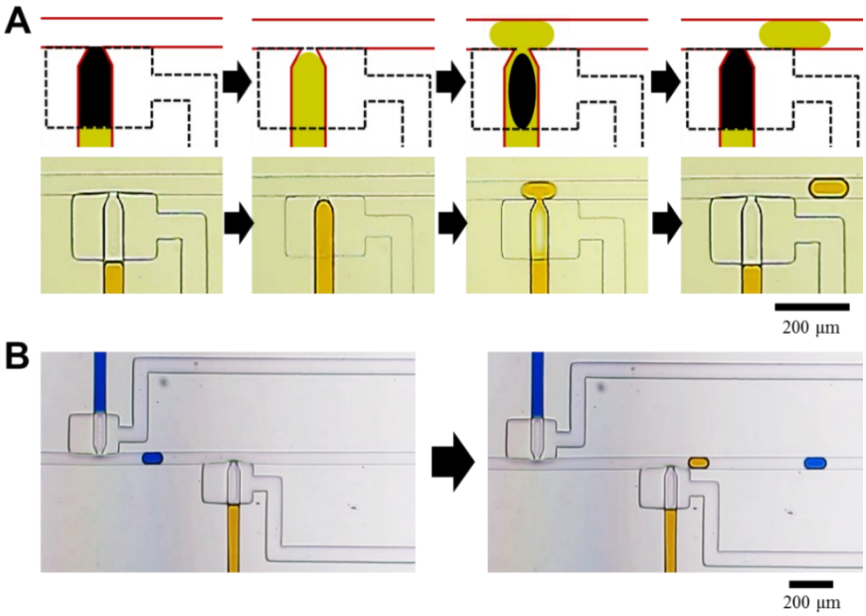

Figure S4. The controlled generation of multi-composition pico-droplets. (A) Schematic diagram for the principle of droplet generation by mechanical separation and time-lapse images of droplet generation. (B) Optical images of two different droplets sequentially formed.

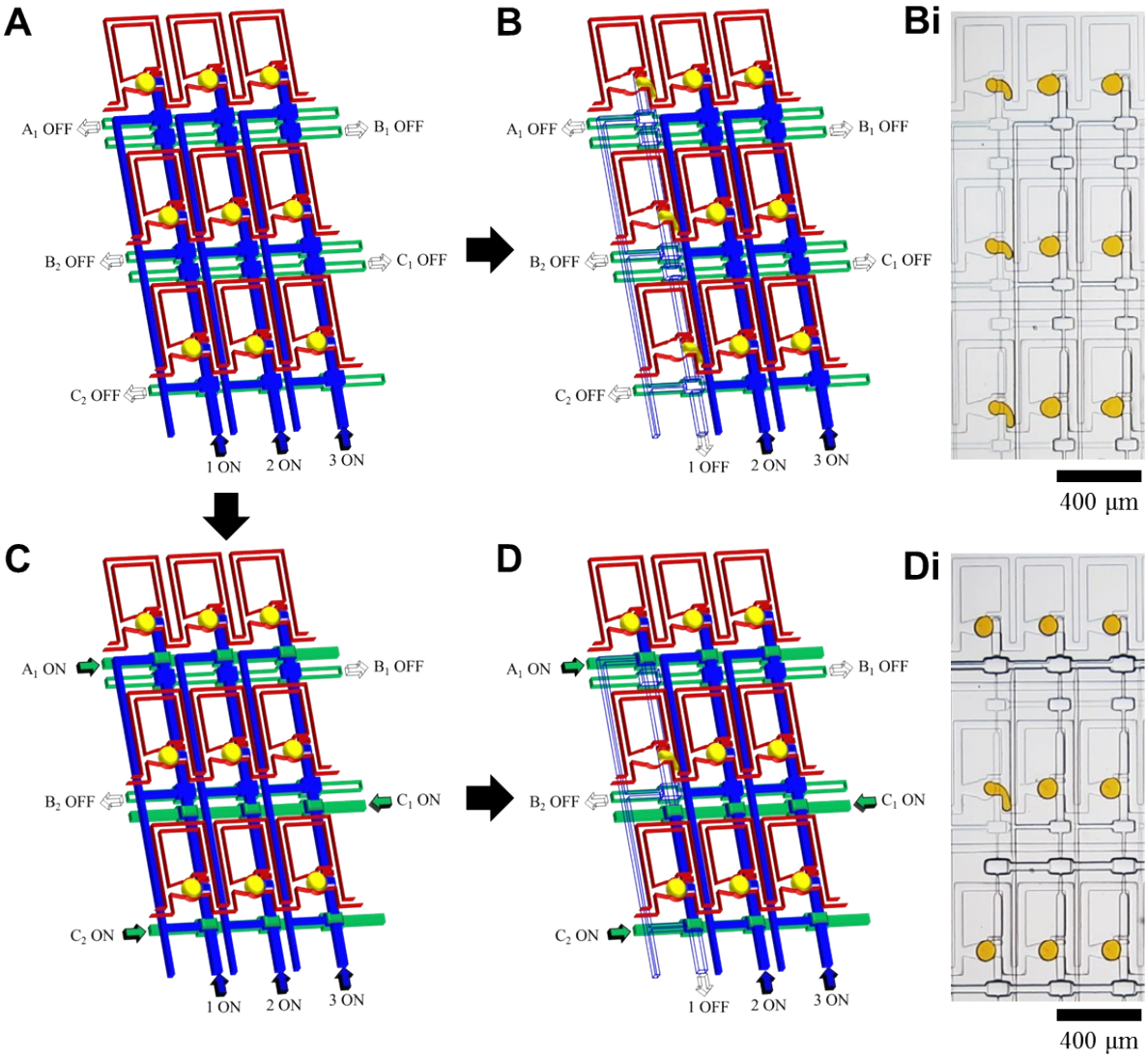

Figure S5. The principle of selective releasing. (A) Droplet trapping using the control valves. (B) Releasing of droplets in the first row by operation of only the control valve and (Bi) its corresponding optical image of result. (C) Operation of block valve C for selective releasing. (D) Selective releasing of only the B2 droplet when control valve 1 is turned off and (Di) its corresponding optical image of result.

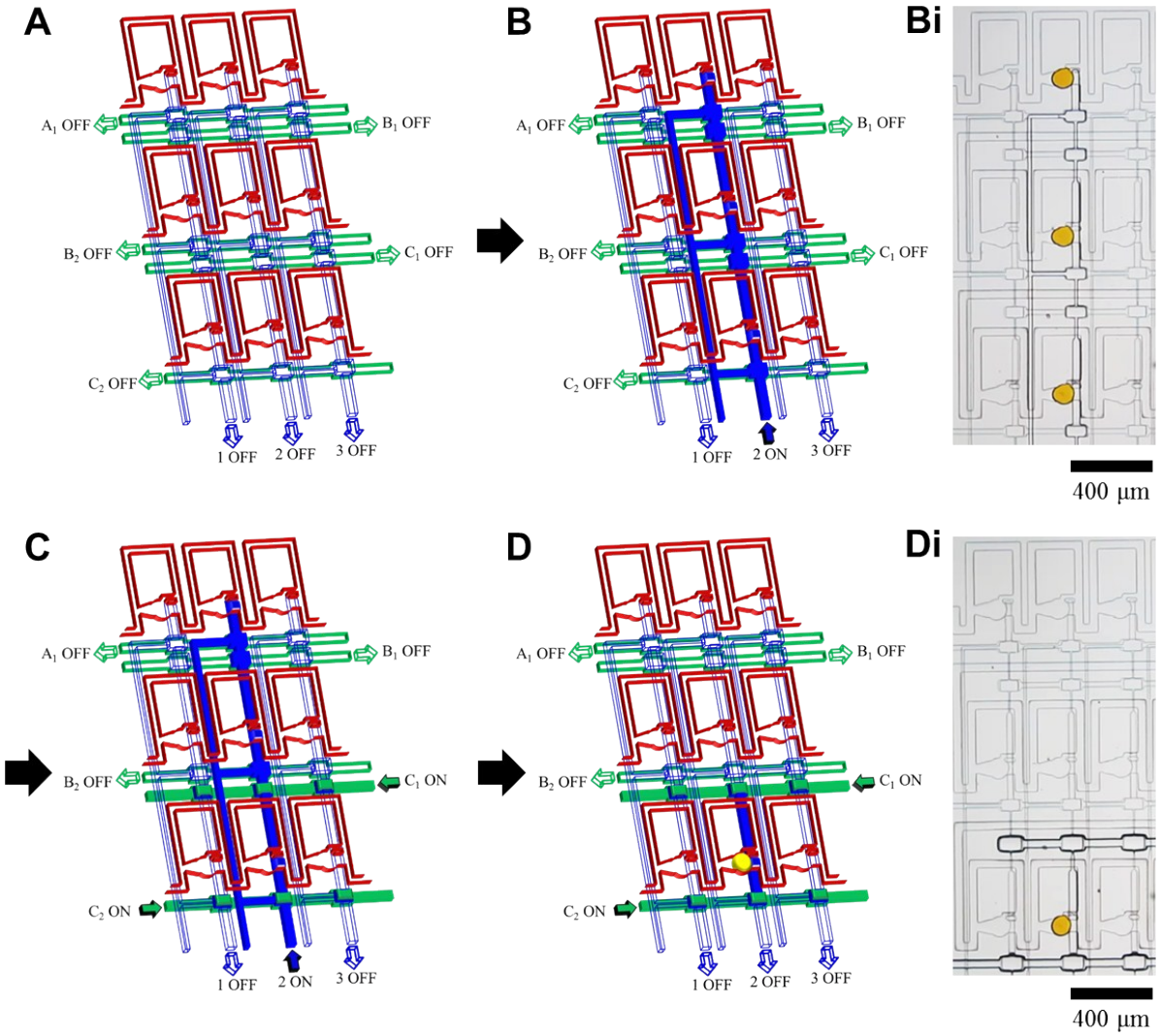

Figure S6. The principle of selective trapping. (A) No trapping of droplets with non-actuated control valves. (B) Trapping of droplets on the second row by the operation of only control valve 2 and (Bi) its corresponding optical image of results. (C) Operation of block valve C for selective trapping. (D) Selective trapping of only the C2 droplet when control valve 2 is turned off and (Di) its corresponding optical image of results.

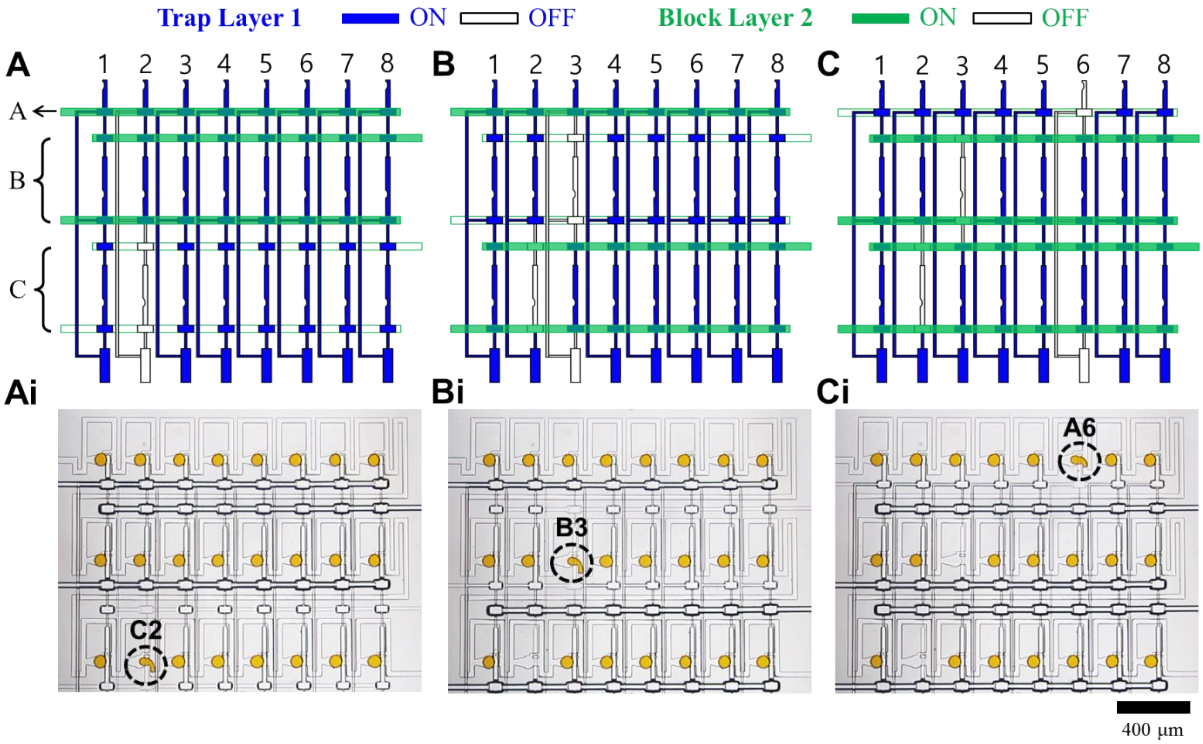

Figure S7. Schematic diagram of the control and block valve state as it corresponds to the selective releasing of desired droplets. The state of control and block valves for sequential releasing of C2 (A), B3 (B), and A6 (C) pico-droplets. Optical images for selective releasing of C2 (Ai), B3 (Bi), and A6 (Ci) pico-droplets.

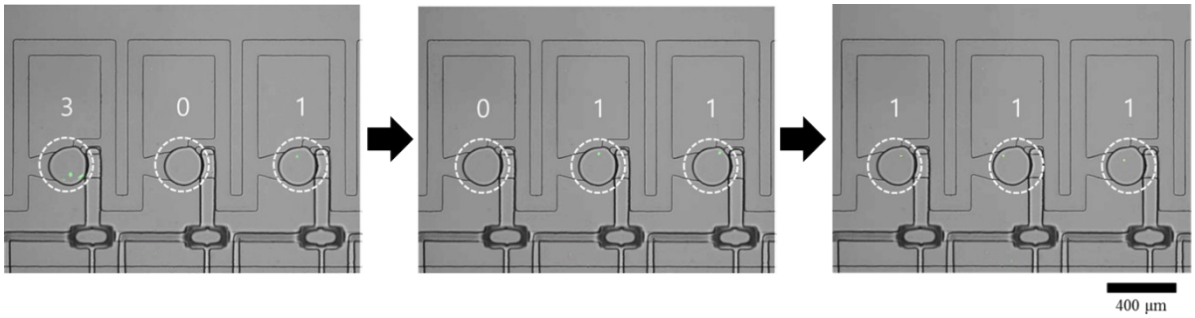

Figure S8. Sequential images showing the single-cell array process. Only single-cell encapsulated droplets are trapped via the repetition of selective trapping and releasing.

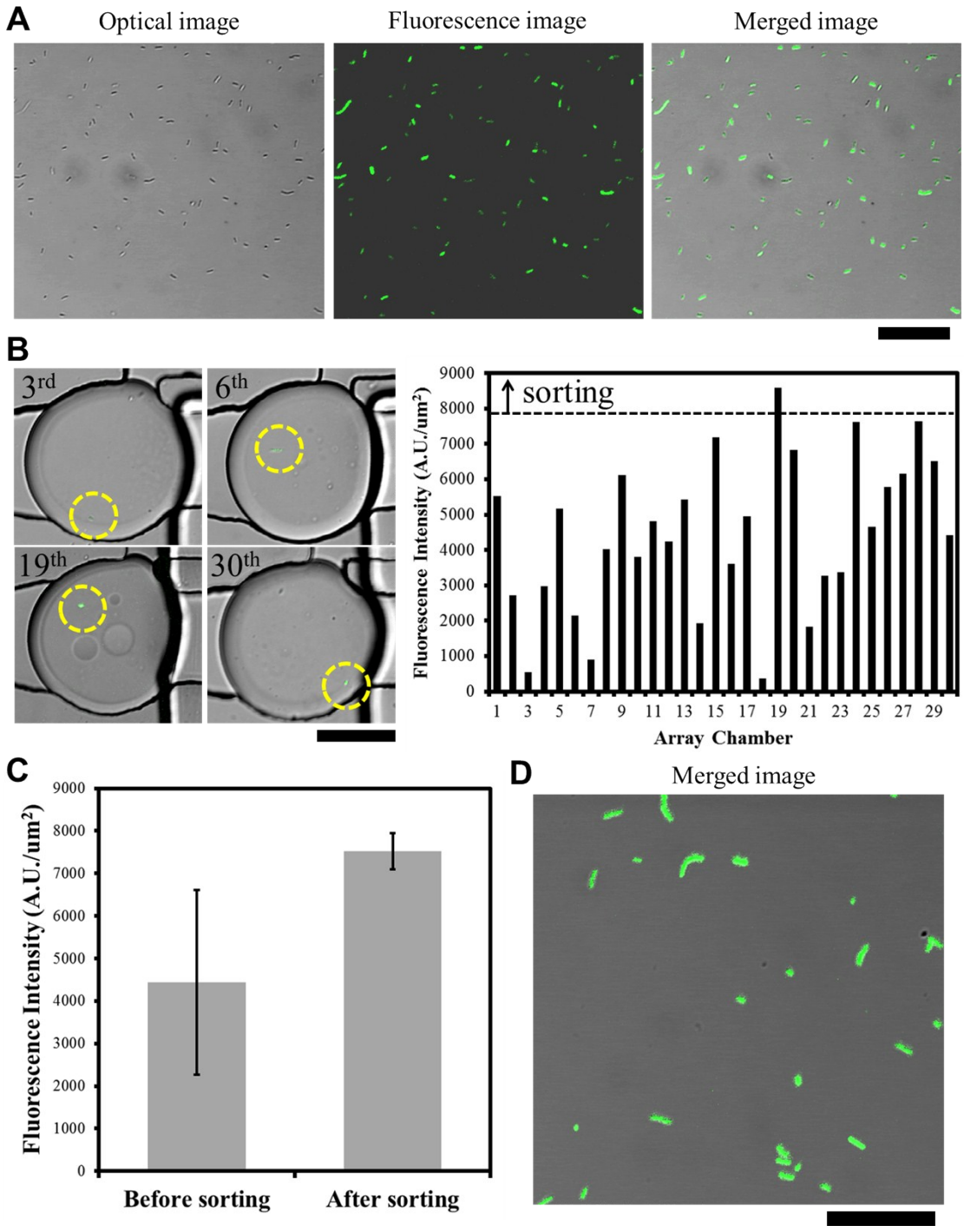

Figure S9. Screening and sorting desired single bacteria from heterogeneous population expressing GFP. A) Heterogeneous GFP expression from genetic engineered *E.coli* MG1655 (pTKU1-12R) response to AHL. B) Single-cell screening by the SDA operation and its GFP expression distribution. C) Comparison of quantitative result between original and sorted population. D) Merged image for homogeneous GFP expression in the individual cells. Scale bars indicate 50 μm.

Movie S1. Selective release of on-demand three droplets from a 2D SDA.

Movie S2. Selective collection of on-demand droplets on a 2D SDA.