Supplementary Information

Pairing Cells of Different Sizes in a Microfluidic Device for Immunological Synapse Monitoring

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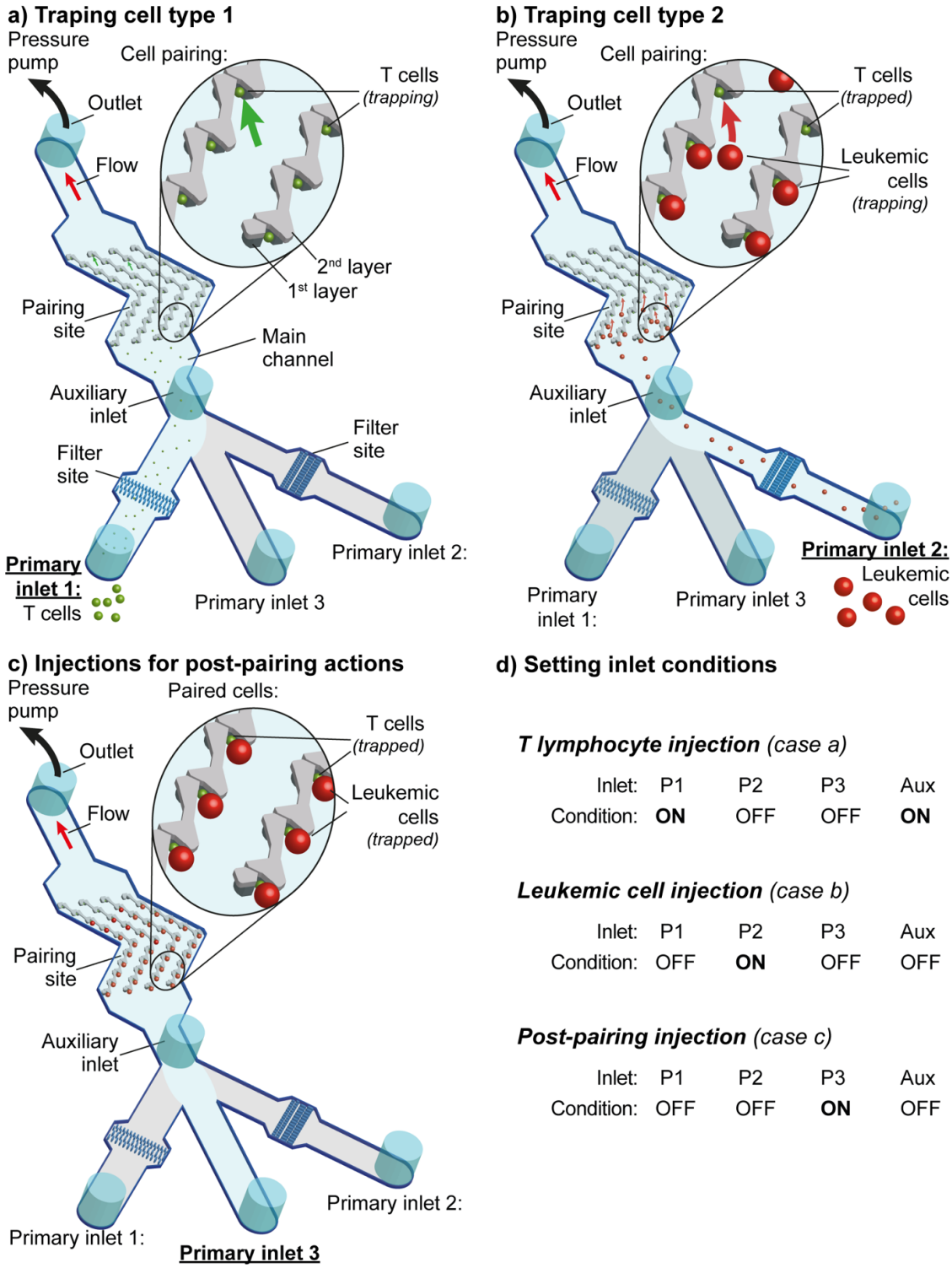

Fig. S1 The inlet flow conditions for three different actions. a) T lymphocytes had dimensions significantly smaller than the channel, and thus, the channel height had to be modified. Hence, T lymphocytes were injected via the primary inlet 1 while keeping auxiliary inlet ON to push cells towards layer 1 for single-cell capturing. b) Leukemic cells fit well with the channel height; thus, no channel height modulation was required. As a result, the primary inlet 2 was used for cell injection while turning the auxiliary inlet OFF. c) The post-pairing actions required additional solutions to be injected. A cell-free inlet, the primary inlet 3, was used to minimize cellular contamination while keeping the auxiliary inlet OFF. d) A summary of the inlet conditions shows the required settings for each case.

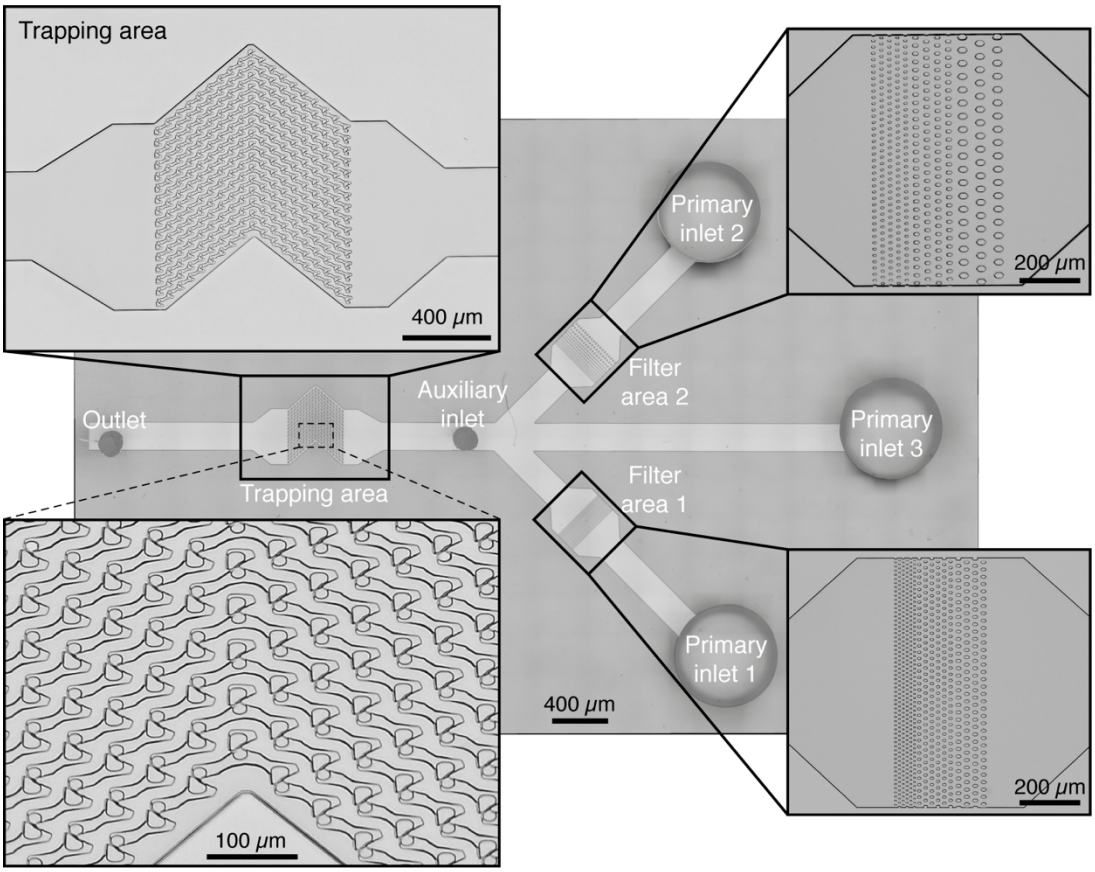

Fig. S2 A view of the device showing the main parts.

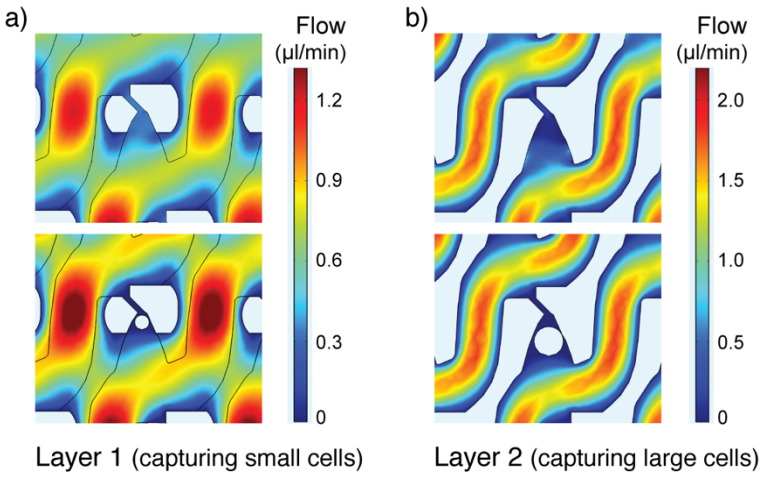

Fig. S3 a) COMSOL simulations show the flow profile around a capture site at layer 1 for small cells. A captured cell blocks the narrow opening and prevents more cells from being captured. The following cell flows around the capture site. b) Similar simulations demonstrate the effect of a large cell captured at layer 2.

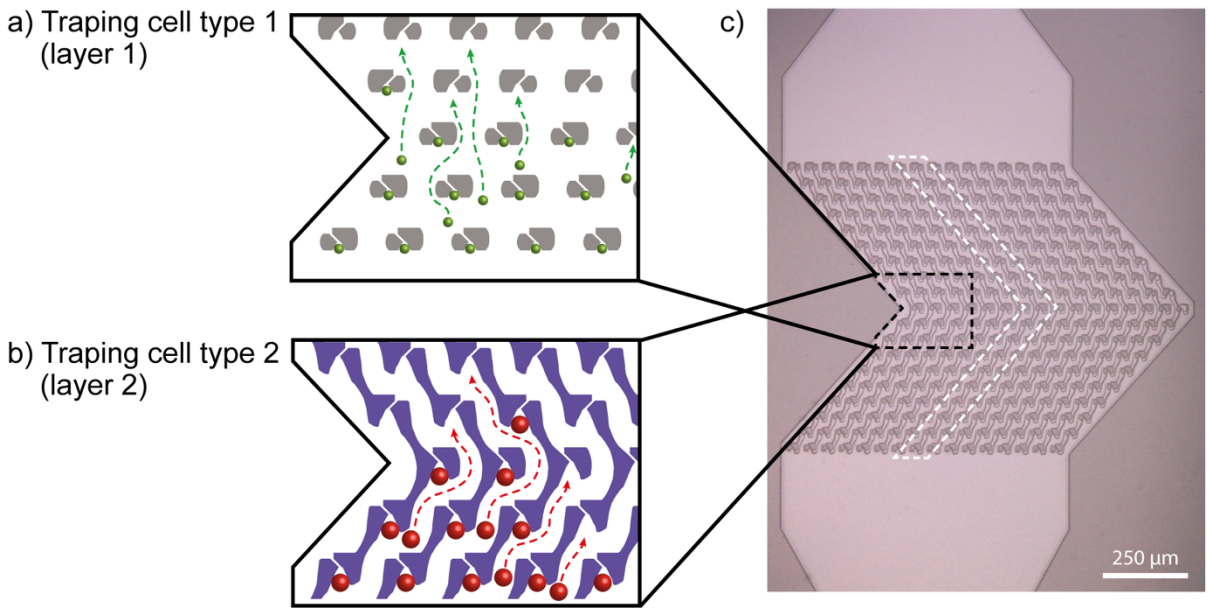

Fig. S4 a) Cell type 1 is trapped at layer 1. b) Cell type 2 follows the >-shaped walls fabricated at layer 2. c) A general view of the trapping area shows both layers as the >-shaped array of pairing traps.

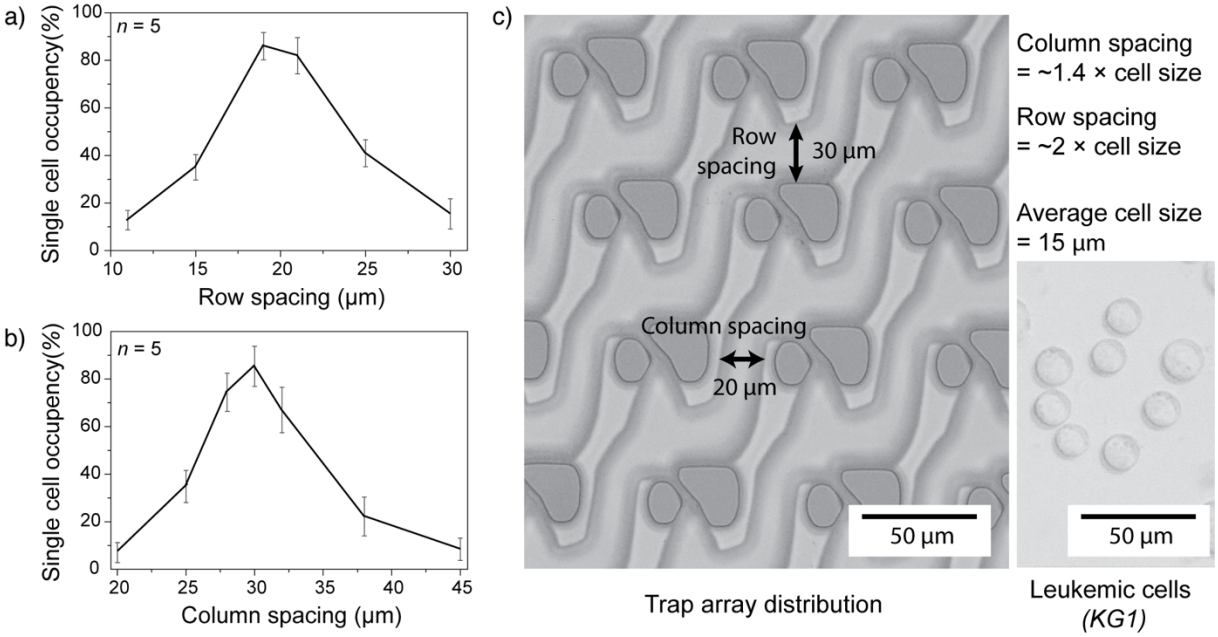

Fig. S5 Different designs were tested experimentally to optimize the pairing site array geometry. a) The row spacing was chosen as 20 µm and b) the column spacing as 30 µm. c) Brightfield images show how the trapping area geometry and larger cells (KG1) compared to each other.

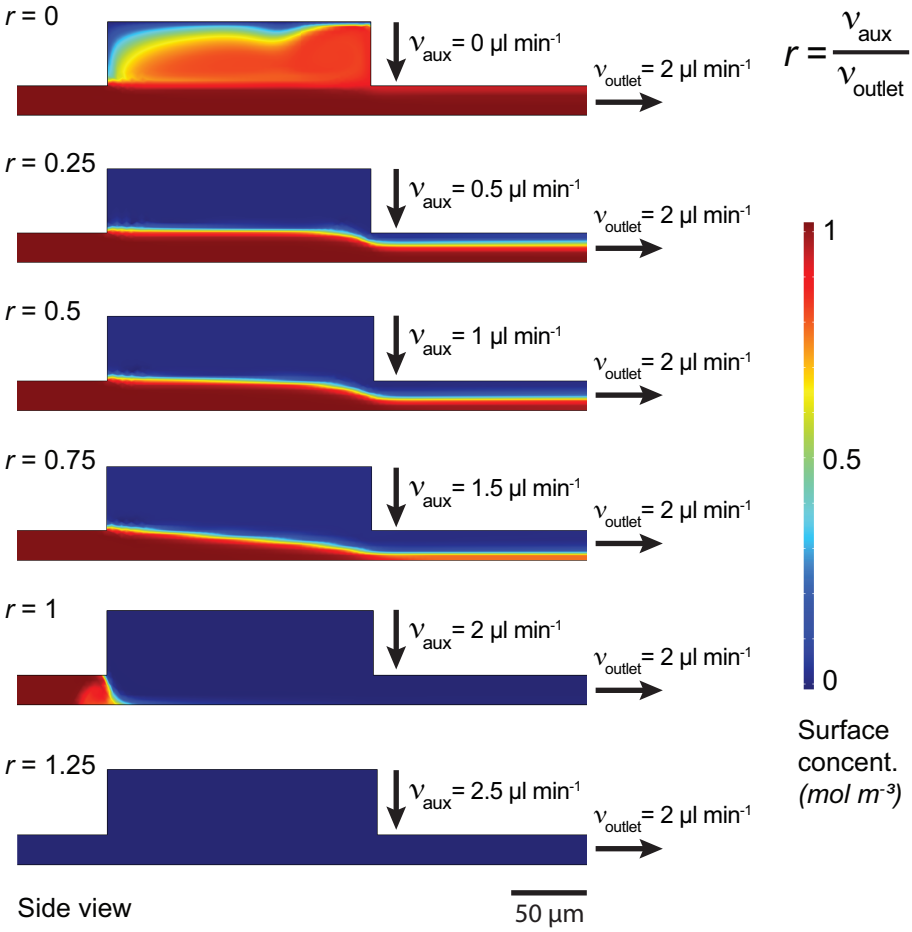

Fig. S6 Simulations show the effect of the auxiliary and outlet flow ratio (*r*). For r = 0, the trap area receives the exact solution injected at the primary inlet. Increasing the flow ratio (*r*) results in a decreased height of the injected solution. When *r* reaches 1, the channel is blocked, and the injected solution cannot reach the trapping area. For *r* > 1, the auxiliary flow pushes the injected solution back. In this case, the trapping area is separated from the inlet area, and the active sub-channel is cleaned.

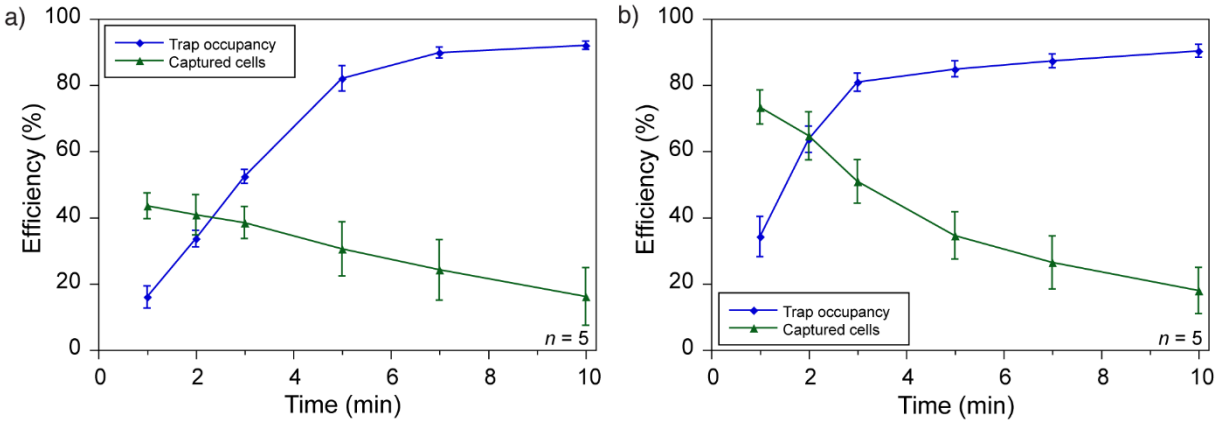

Fig. S7 a) The captured cell efficiency (captured cells per all cells injected in the channel) of T lymphocytes is relatively lower as the trap design was done according to the larger cells, *e.g.*, KG1 and blasts. Around 30% of the T lymphocytes could be captured to reach a single-cell trap occupancy level of 80% in 5 minutes. b) As the trap geometry in the array was designed according to KG1, a higher cell capturing efficiency (>50%) was obtained when compared with T lymphocytes to reach a single-cell trap occupancy level of 80% in a shorter time (3 minutes).

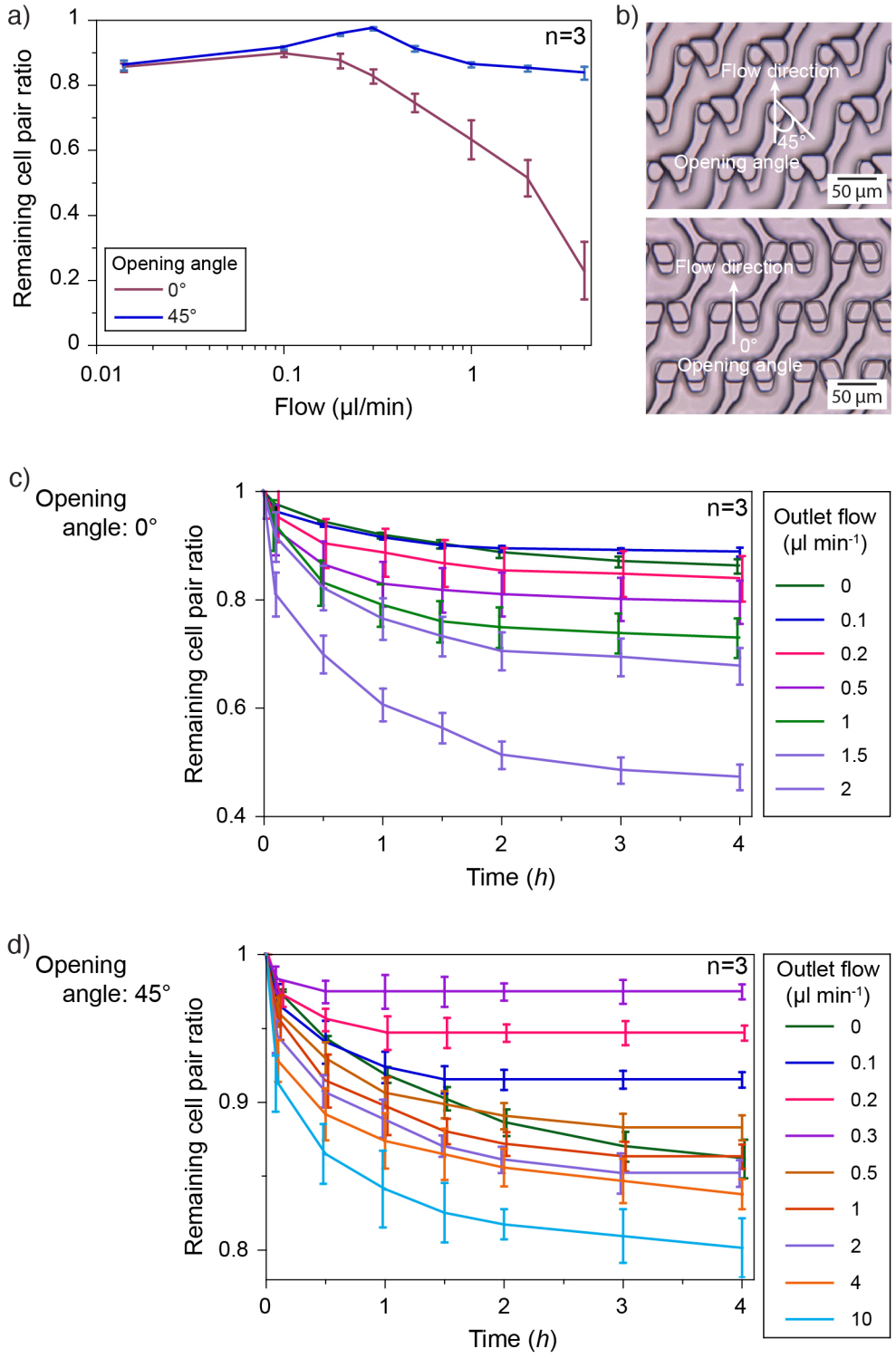

Fig. S8 a) Long incubation performance of the method was demonstrated with the remaining cell pair ratio (*i.e.*, the number of cell pairs at time *t* by the number of cell pairs at t = 0) for (b) two different trap geometry. The performance was tested at t = 0.5, 1, 1.5, 2, 3, and 4 h for an opening angle of (c) 0˚ and (d) 45˚.

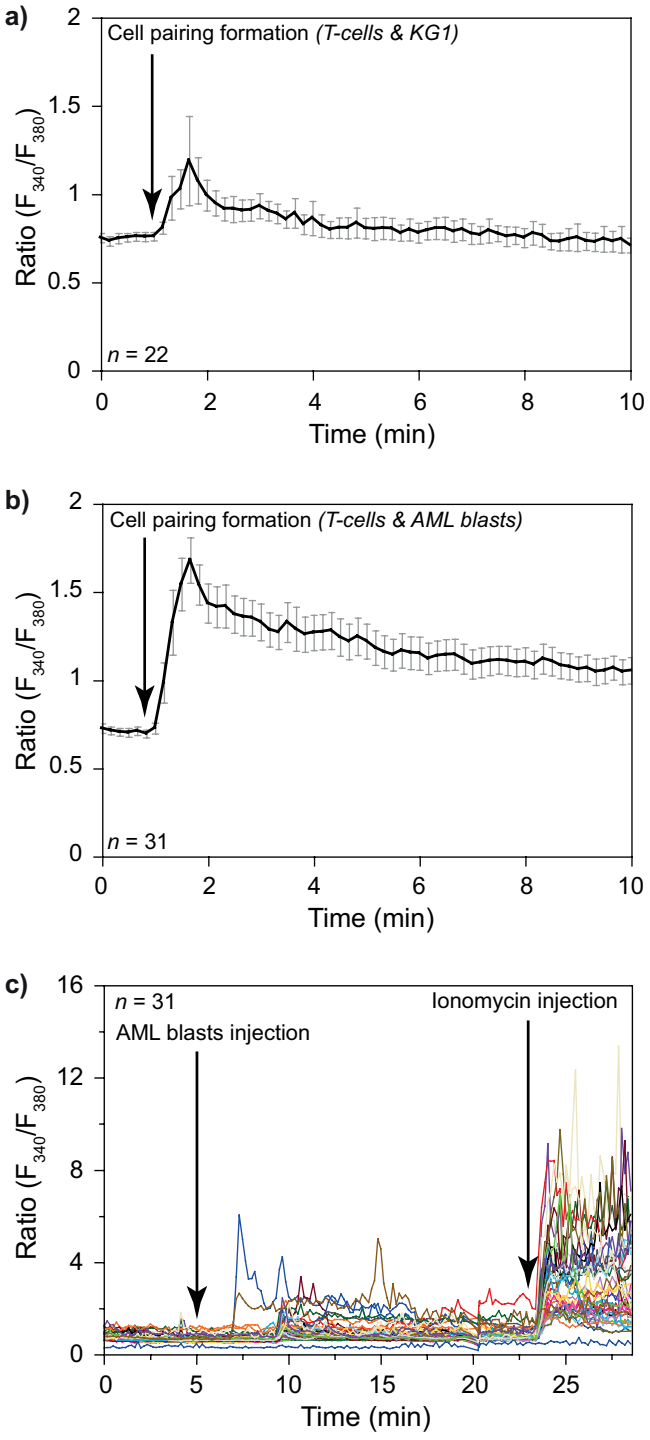

Fig. S9 Other demonstrations of the Ca^{2+} imaging experiments showed in Figure 5. (a) corresponds to T lymphocytes - KG1 pairs and (b) corresponds to pairs of T lymphocytes and AML blasts from healthy donors. Error bars correspond to the standard error. c) An allogenic condition was obtained by pairing primary human $CD8^{+}$ T lymphocytes from healthy donors and primary AML blasts. Positive control was performed by adding ionomycin at the end of the experiment to check T cells viability and responsiveness to stimuli.

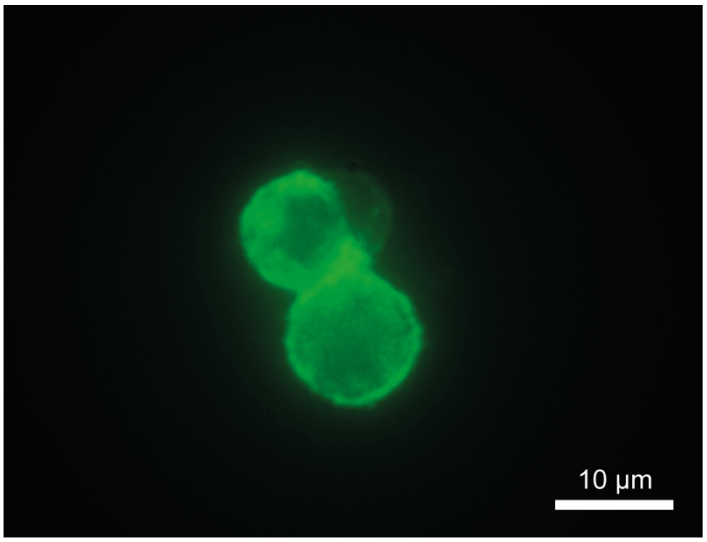

Fig. S10 A demonstration of post-pairing actions where cells were fixed, permeabilized, and had actin filaments stained with Phalloidin-488.