Supporting Information

Microfluidic Device for Negative Enrichment of Target Cells by Affinity Chromatography

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	Outlet 1	Outlet 2	Outlet 4	Outlet 6
0min	16.0	8.0	4.0	1.0
10min	13.0	2.0	0.0	0.0
20min	22.0	4.0	5.0	1.0
30min	90.0	3.0	2.0	0.0
40min	54.0	10.0	4.0	1.0
50min	73.0	5.0	3.0	2.0
60min	54.0	4.0	1.0	2.0

Table S1 Exact cells counts at different bottom channels outlet over time.

	Outlet 1	Outlet 2	Outlet 4	Outlet 6	Spike with MEC sample 1	Spike with MEC sample 2
10 min	13	2	0	0	29	54
30 min	90	3	2	0	24	44

Table S2 Exact cells counts at different bottom channels with/without adding mouse endothelial cells.

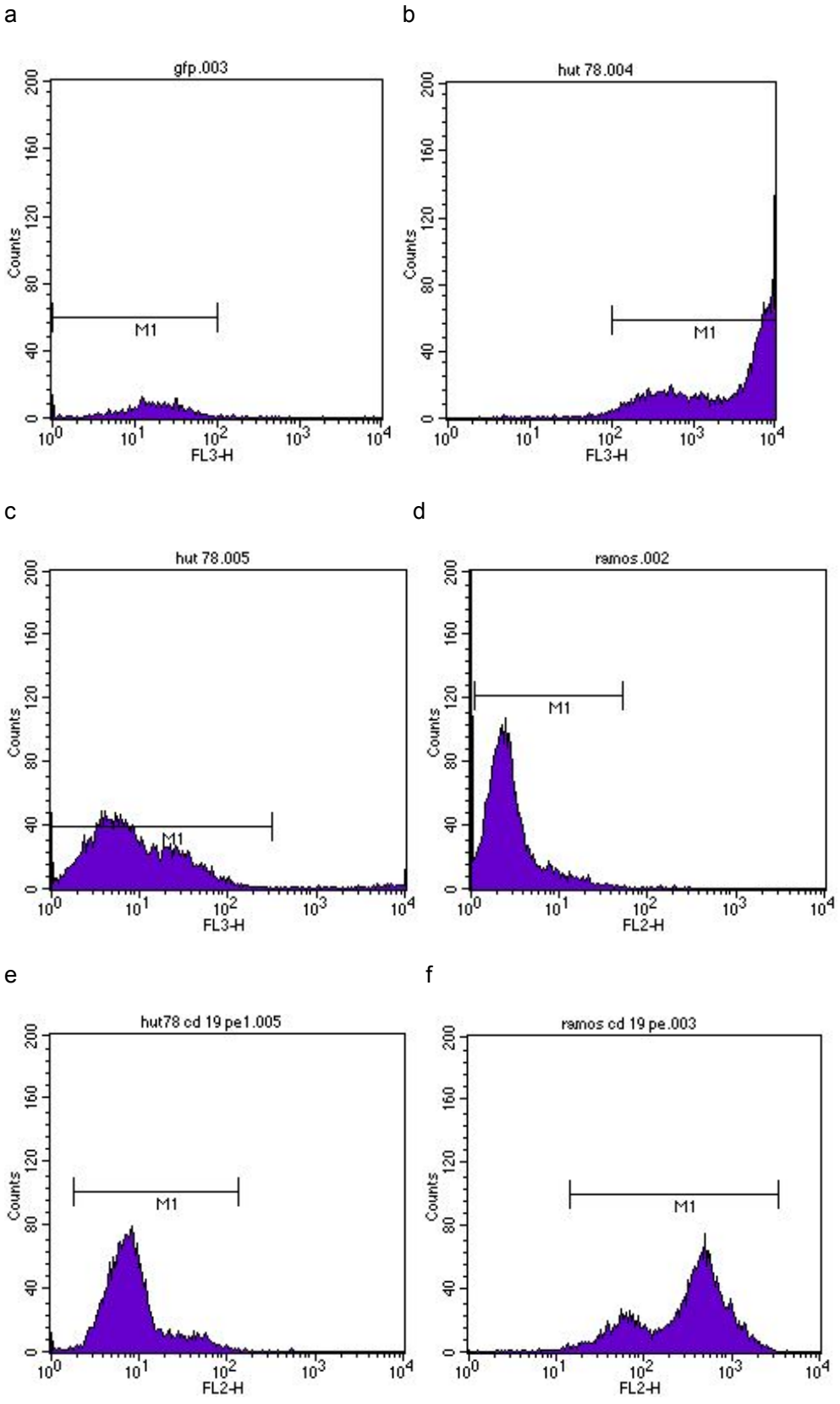


Fig. S1 Cells surface antigen determination by flow cytometry. a) C166-GFP cells

stained with biotin conjugated anti-CD71 and streptavidin conjugated PE-647. b) Hut78 cells stained with biotin conjugated anti-CD71 and streptavidin conjugated PE-647. c) Unstained Hut78 cells. Staining Protocol: Cells suspension in 200μL PBS solution was first incubated with 20 μL biotin conjugated anti-CD71 (6.25 μg/mL) for 30 min at room temperature. Cells mixture was then centrifuged at 4500 rpm for 4 min. After removing supernatant, cells were resuspended with 200 μL PBS and incubated with 1μL streptavidin conjugated PE-647 (1 mg/mL, Invitrogen) for 30 min at room temperature. Cells sample was then washed twice with 500μL PBS and loaded into BD FACS Calibur flow cytometer. d) Unstained Ramos cells. e) Hut78 cells stained with PE labeled anti-CD19. f) Ramos cells stained with PE labeled anti-CD19. Staining protocol is similar to previous anti-CD71 protocol. The difference is the use of PE labeled anti-CD19 instead of biotin conjugated anti-CD71 and streptavidin conjugated PE-647.

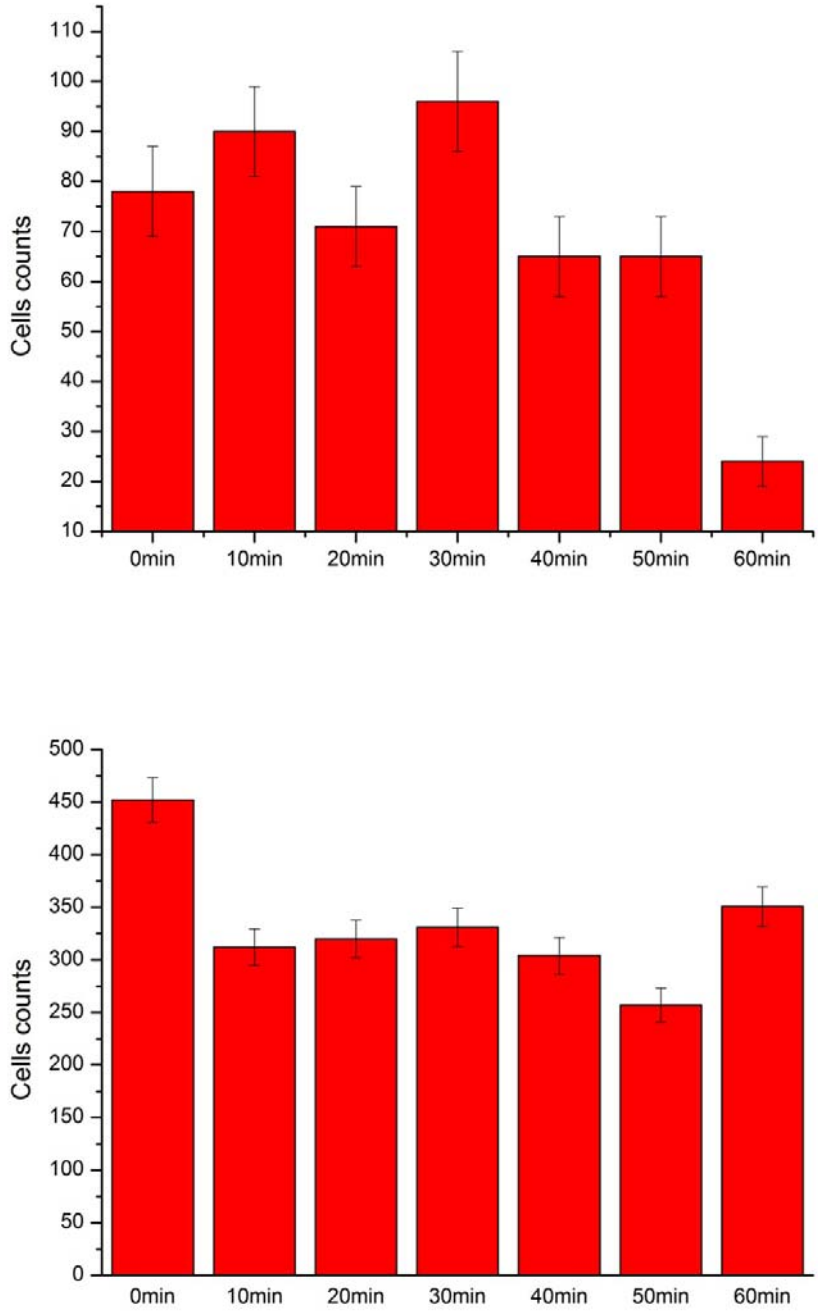


Fig. S2 Cells counts at device inlet. a) Hut78 cells were loaded into the non-coated device at 0.05 mL/h with concentration of 1460±120 cells/μL. Two 30 seconds videos were recorded at each time point. Cells counts is the

number of cells passed the inlet in 30 seconds. b) Ramos cells were loaded into the non-coated device at 0.05 mL/h with concentration of 5150±244 cells/μL. Two 30 seconds videos were recorded at each time point. Cells counts is the number of cells passed the inlet in 30 seconds. Error bar represents counting error.

Fig. S3 Schematic of parallel separation device. Grey area is the multi perpendicular channel separation units, featuring 5 bottom inlets and channels. Main loading channel is 7.62 mm in length, 1 mm in width and 40 μm in height. Two branch channels are same in dimension (3.81 mm *1 mm *40 μm, l*w*h).

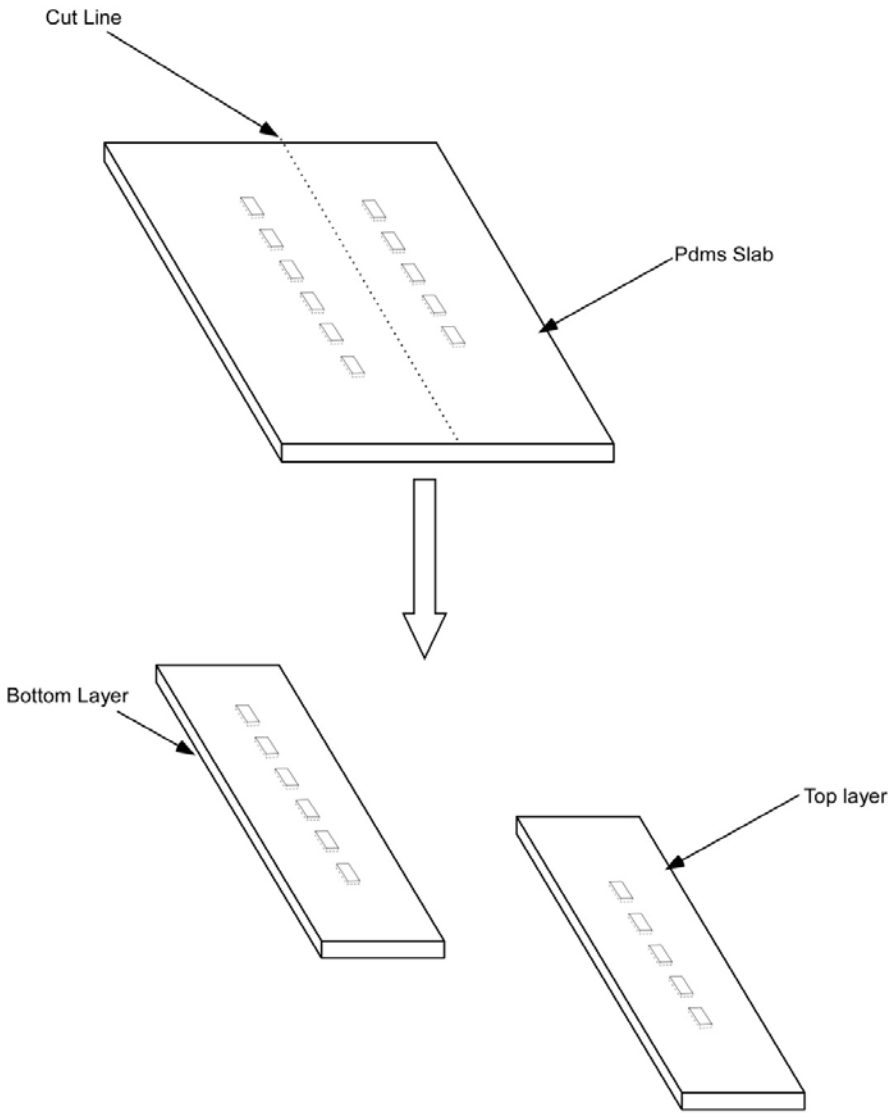
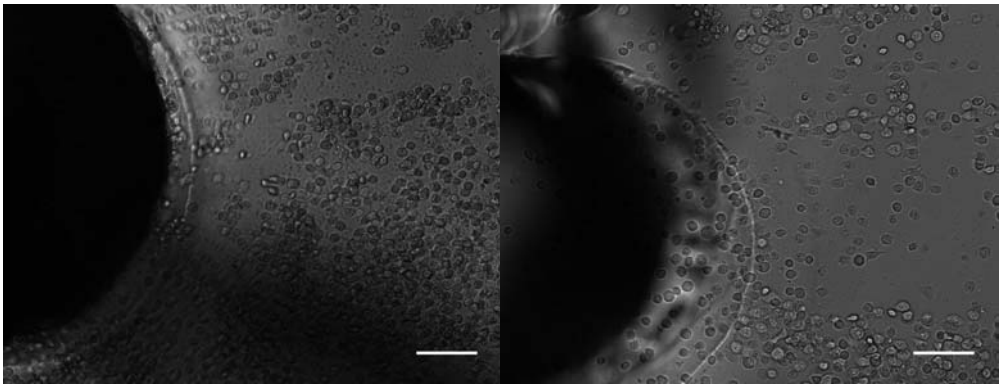


Figure S4. Fabrication of bottom and top layer.

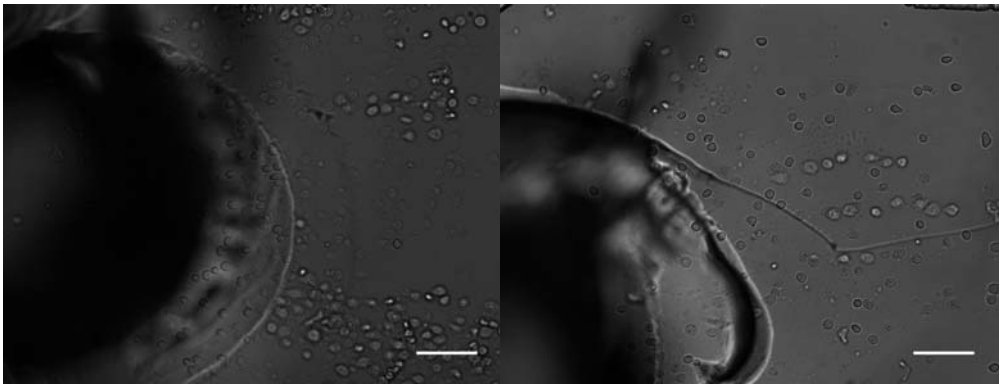
a

b



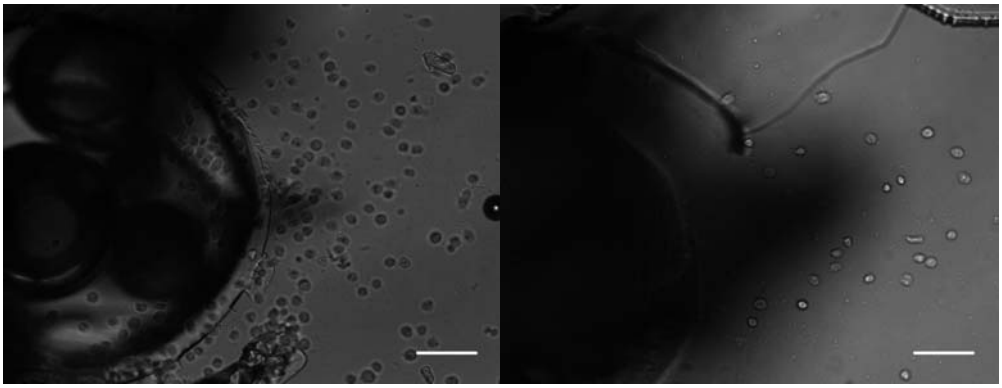
c

d



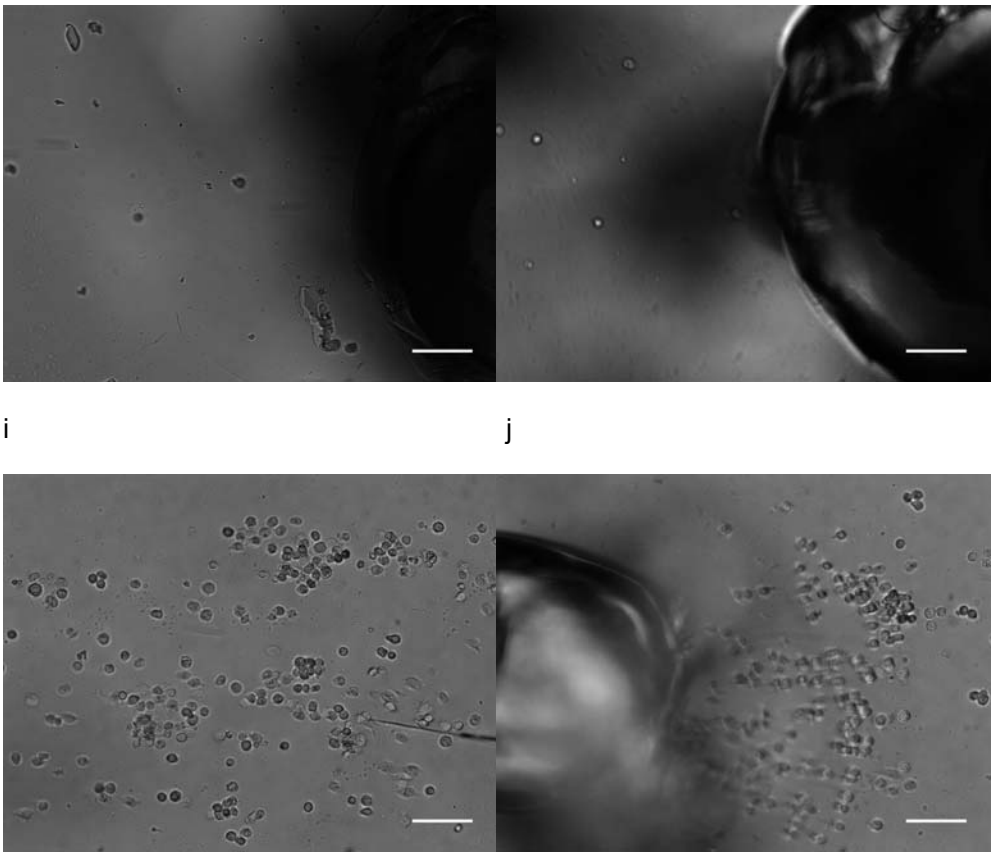
e

f



g

h



i j

Fig. S5 Actual images of surface capture Hut-78 cells. a)-f) showed cells capture at bottom channel inlet 1, 2, 3, 4, 5, 6, respectively. Decreased cells capture can be observed, since fewer cells would reach the later bottom channel. g) and h) represented cells capture at bottom channel outlet 5 and 6, respectively, indicating no significant capture enhancements at outlet area. i) and j) showed cells capture at top channel 1 and top channel inlet 1, respectively. Scale bar is 100 μm.

Enhanced cell capture at inlet area

At the vertical inlet area, the connecting tube has a larger cross section area, which leads to lower cell velocity than the separation channels. When cells first contact theh

affinity surface, the slow flow rate promotes the enhanced cell capture. Also, dead volume caused by the interface geometry also enhances cell capture as cells traveled slowly in these areas. In addition to slow flow rate, vertical direction of hydrodynamic force will also push cells to interact with surface and increase the possibility of cell capture.

Video S1 Cells were being captured at second perpendicular channel under 0.05 mL/h flow rate. Only Hut-78 T cells (660 cells//μL) were loaded into the device.

Video S2 Cells were passing through outlet 1 at 20 minute under 0.05 mL/h flow rate. All cells are Hut-78 T cells (660 cells/μL).

Video S3 Cells were passing through outlet 6 at 20 minute under 0.05 mL/h flow rate. Small particles were cell debris not intact cells. Only Hut-78 T cells (660 cells/μL) were loaded into the device.

Video S4 Cells mixture was passing through outlet 6 at 20 minute under 0.05 mL/h flow rate. Fluorescence video was recorded. Hut-78 T cells spiked with mouse endothelial cells (1080 cells/μL, 1:1 ration) were loaded into the device.