Supporting Information

## Enhanced Directional Cell Migration Induced by Vaccinia Virus on A Microfluidic-based Multi-shear Cell Migration Assay Platform

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**Fig. S1** Different width regular exclusion zones could easily be realized by simply controlling width-variable microvalves on designed microfluidic platform. Scale bars: 100 μm.



Fig. S2 The fluorescent streak images of 7  $\mu$ m beads in six parallel channels with inlet velocity of 0.06 m/s. Scale bars: 100  $\mu$ m.



Fig. S3 The simulation results of the distribution ratio of steady velocity in six parallel channels when inlet velocity ranged from  $10^{-4}$  m/s to 0.3 m/s.



**Fig. S4** Time-lapse images of single migratory cell tracking at different MOI of VACV. (A)-(C), the lower rows were fluorescence field images. Scale bars: 50 μm.



**Fig. S5** The schematic view of integrated microfluidic platform in two working mode. (A) Cell loading mode: cells suspension was injected from the inlet when microvalves were actuated. (B) Perfusion mode: used for the steps of virus infection and shear stress application.

**Table S1** The steady velocity and the ratio of steady velocity in six parallel channels with variation of inlet velocity from  $10^{-4}$  m/s to 0.3 m/s. These results came from simulation.

Inlet	Velocity in the channels $V_x$ (m/s)						Ratio of velocities in the channels and inlet $(V_x/V_0)$					
velocity	V	V	V	V	V	V	V /V	V AV	V AV	V /V	V AV	VA
V <sub>0</sub> (m/s)	• 1	<b>v</b> <sub>2</sub>	• 3	¥ 4	<b>v</b> 5	* 6	* 1/ * 0	v 2/ v 0	¥ 3/ ¥ 0	* 4/ * 0	¥ 5/ ¥ 0	¥ 6/ ¥ 0
10-4	2.82E-5	4.52E-6	8.46E-7	8.49E-7	4.53E-6	2.82E-5	0.282	4.52E-2	8.46E-3	8.49E-3	4.53E-2	0.282
10-3	2.82E-4	4.53E-5	8.48E-6	8.49E-6	4.53E-5	2.82E-4	0.282	4.52E-2	8.48E-3	8.49E-3	4.53E-2	0.282
10-2	2.82E-3	4.52E-4	8.51E-5	8.48E-5	4.53E-4	2.82E-3	0.282	4.52E-2	8.51E-3	8.48E-3	4.53E-2	0.282
0.1	2.81E-2	4.54E-3	8.53E-4	8.46E-4	4.52E-3	2.83E-2	0.281	4.54E-2	8.53E-3	8.46E-3	4.52E-2	0.283
0.2	5.59E-2	9.10E-3	1.71E-3	1.69E-3	8.99E-3	5.66E-2	0.280	4.55E-2	8.55E-3	8.45E-3	4.50E-2	0.283
0.3	8.42E-2	1.37E-2	2.57E-3	2.53E-3	1.34E-2	8.52E-2	0.280	4.56E-2	8.57E-3	8.43E-3	4.48E-2	0.284

## Movie S1

Directional cell migration induced by VACV in dynamic condition. Cells (MOI = 1) were applied to shear stress of 15 dyne cm<sup>-2</sup> at 10 hpi for 12h. The fluorescence of GFPs expressed in the cytoplasm was used to ensure the cells to be infected. The dot white lines represented the boundaries of wound. Infected cells tended to migrate along the direction of fluid flow in presence of shear stress. Scale bars: 100  $\mu$ m.

## Movie S2

Random cell migration induced by VACV in static condition. Cells (MOI = 1) were monitored at 10 hpi for 12h without shear stress. The fluorescence of GFPs expressed in the cytoplasm was used to ensure the cells to be infected. The dot white lines represented the boundaries of wound. Infected cells migrated randomly to wound areas. Scale bars: 100  $\mu$ m.