Supplementary Materials for Huang *et. al.* manuscript

Movies

Each of the following movies contains a series of 96 images, with 5 minutes interval and a total of 7.5 hrs duration. The movie is post processed using ImageJ, and played at 15 fps. Each frame size is 480 × 360 pixels or 309.9 × 232.2 μm.

1. Smovie 1: MDA-MB-231 cells embedded in collagen matrix in the absence of the flow.
2. Smovie 2: MDA-MB-231 cells embedded in collagen matrix in the presence of the flow (flow direction: from right to left).
3. Smovie 3: Single MDA-MB-231 cell migrating in an amoeboid mode.
4. Smovie 4: Single MDA-MB-231 cell migrating in a mesenchymal mode.

Figures

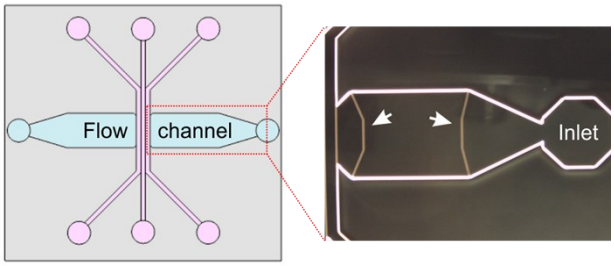

Figure S1: Schematic of the microfluidic device containing contact lines (cross section size of 10 µm × 5 µm) as indicated by the white arrows on the surface of the horizontal flow channel. These special contact lines are designed to avoid trapping bubbles when flow is initially introduced into the flow channel inlet.

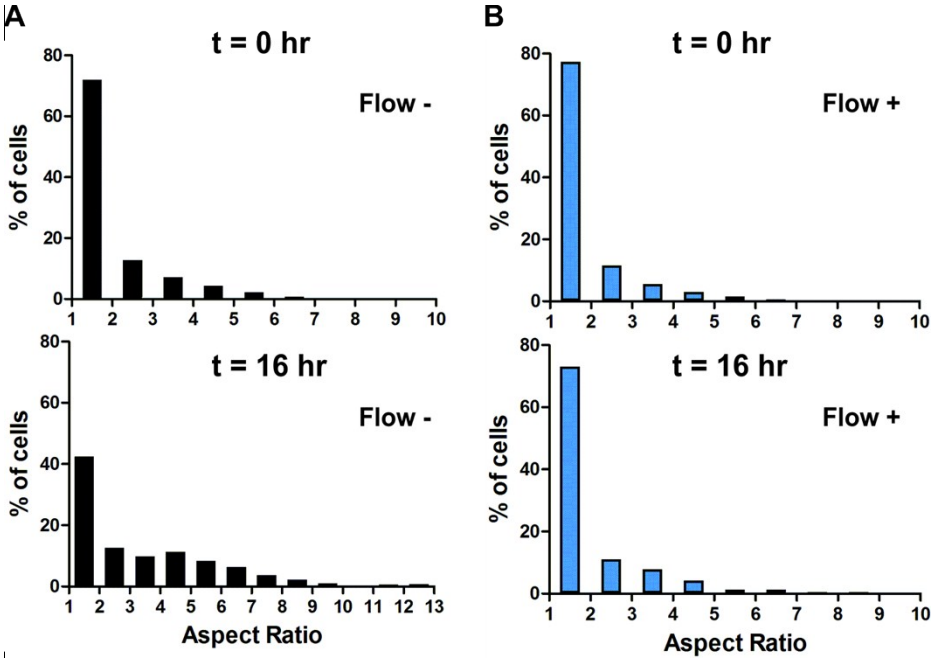

Figure S2: Histograms of cell aspect ratio at t = 0 hr and t = 16 hr in the absence (A) and presence (B) of interstitial flows of 2 μm/s of three independent experiments (including the one shown in Fig. 2 of the main text). 600 cells are analyzed in each condition.

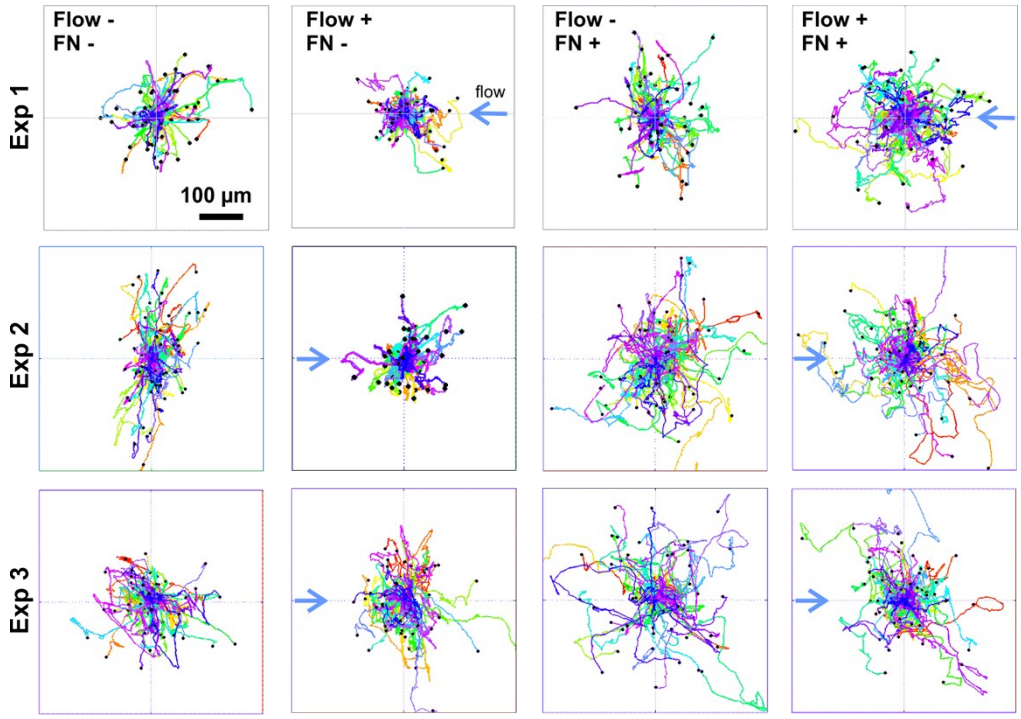

Figure S3: Cell trajectories from three independent experiments. Each colored line is a cell track of 16 hours duration. 60 cells were tracked in each condition. Flow rate is 2 μm/s, and FN concentration is 100 $\mu g/mL$.

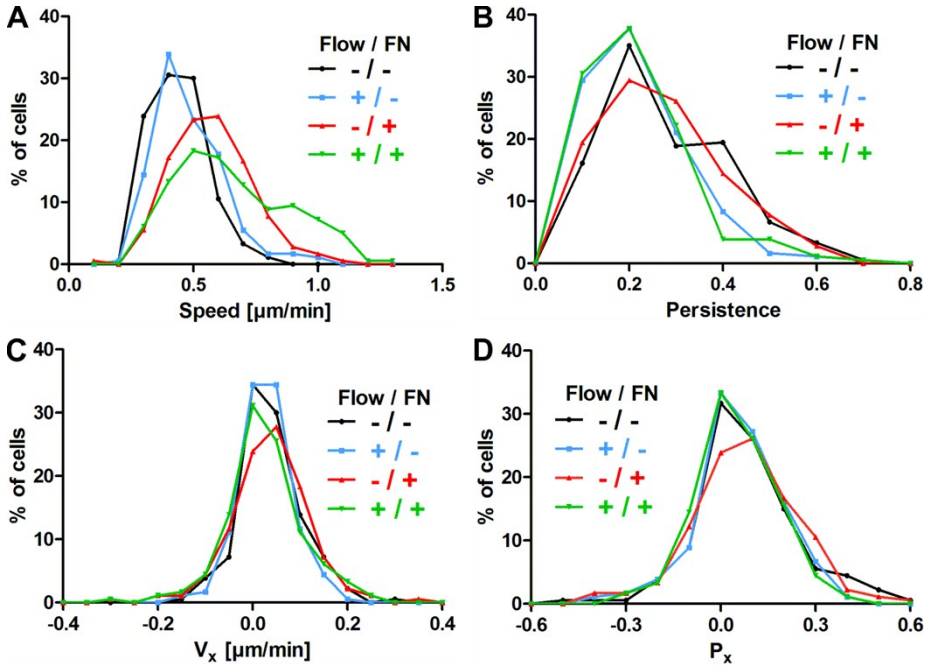

Figure S4: Histograms of cell motilities parameters: (A) average speed; (B) migration persistence (C) cell velocity along the flow direction; (D) persistence along the flow direction under four flow and FN conditions. Each plot represent a combined cell population of three experiments.

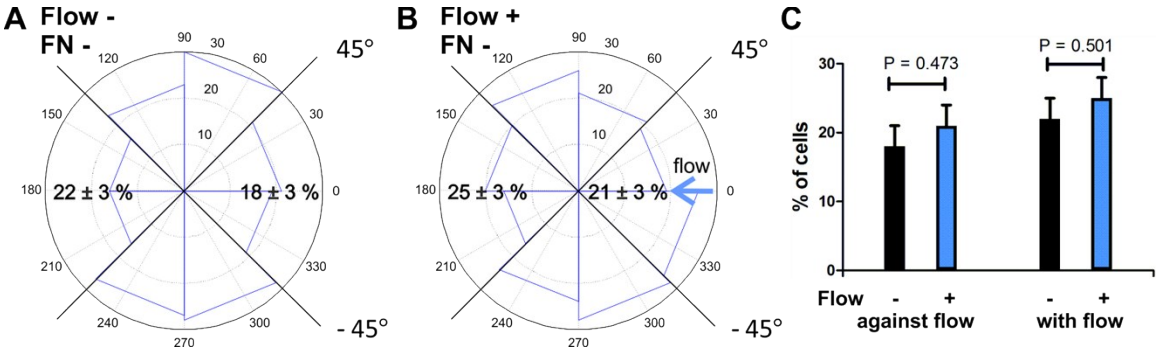

Figure S5: Polar histograms indicated cell migration direction in the absence (A) and presence (B) of flows. (C) Percentage of cells migrate along/against the flow in the absence/presence of the flow. Error bars represent the standard error. Here, if cells migrate within 45° with respect to the flow direction, cells are considered migrating along/against flow direction. The percentages at the left and right quarters indicate the percentage of cells migrate along or against the flow direction, respectively. Each polar plot shows three combined independent experiment results.

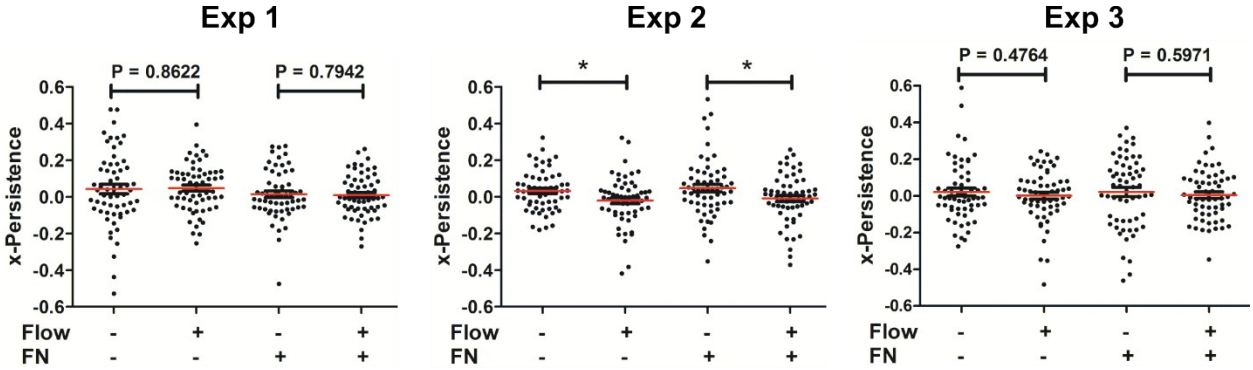

Figure S6: Average cell migration persistence in the flow direction (x-persistence) for three repeated independent experiments.

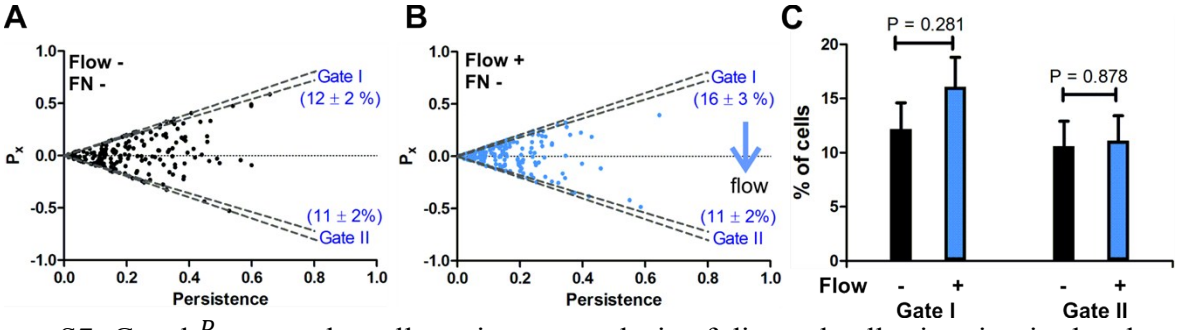

Figure S7: Gated $P_x$versus the cell persistence analysis of directed cell migration in the absence (A) and presence (B) of flow. The gated criteria is that P_x is above 90% of the average persistence (or within 26° with respect to the flow direction) for cells within Gate I and II. Each plot shows the combined cell data points (a total of 180) from three experiments. Flow direction is downward. (C) Percentage of cells migrate against (Gate I) or along (Gate II) the flow. Error bars represent the standard error.

		Polacheck *et. al.* (2011)	Haessler *et. al.* (2012)	Huang *et. al.*

		PNAS	Integrative Biology	This manuscript
Flow experiment set up	Cell Line	MDA-MB-231	MDA-MB-231	MDA-MB-231
	Cell Density	High: 2.5 × 10^5 cells/mL Low: 0.5 × 10^5 cells/mL	2.5 × 10^5 cells/mL	1.0 × 10^6 cells/mL
	Flow Speed	0.3 and 3.0 μm/s	10 μm/s	2.0 μm/s
	Flow Generation	40 Pa Pressure head	Peristaltic pump	Syringe pump
	3D ECM	2.0 mg/mL type I collagen (PH=8.9)	1.5 mg/mL type I collagen with 10%matrigel (PH=7.4, private communication)	1.5 mg/mL type I collagen (PH=7.4)
	Pre-incubation	overnight incubation	overnight incubation	no pre-incubation
	Medium supplement	10 ng/mL EGF	no additional supplement	no additional supplement
	Imaging	15min interval for 16-24 hr	15min interval for 16 hr	5min interval for 16 hr
Results and interstitial flows effect	Average cell speed in static condition	0.1 μm/min	0.17 μm/min	0.36 μm/min
	Motile cell speed gate	one cell diameter in 8hr	0.02 μm/min	0.2 μm/min
	Motility of entire cell population	No significant effect	flow increases population of motile cells	-
	Average cell speed/velocity	no significant effect on average velocity	flow increases average speed, also reflected on a histogram plot	flow enhances the normalized average speed
	Persistence or directionality	flow increases directionality from 0.39 to 0.63	no significant effect on overall average persistence (~0.45)	flow decreases persistence (~0.2)
	Average x-velocity	cells prefer to migrate along the streamline	no significant effect on overall average x-velocity	no significant effect
	Directed velocity/directional migration (within 45° of x-axis)	high cell density: cells migrate against the flow; low cell density: cells migrate along with the flow	flows increase the percentage of cells migrate along with the flow direction	no significant effect on directional migration was observed
	Directed persistence (P^x)	-	no significant effect on overall average P^x; flow increases the percentage of cells (5-10%) with P^x both along and against the flow direction	no significant effect on overall average P^x and directed P^x in the flow direction was observed
	Morphology	-	-	flow enhances amoeboid phenotype

Table S1: Summaries of the experimental settings and results from three labs on the roles of interstitial flows on cell motility using microfluidic models.

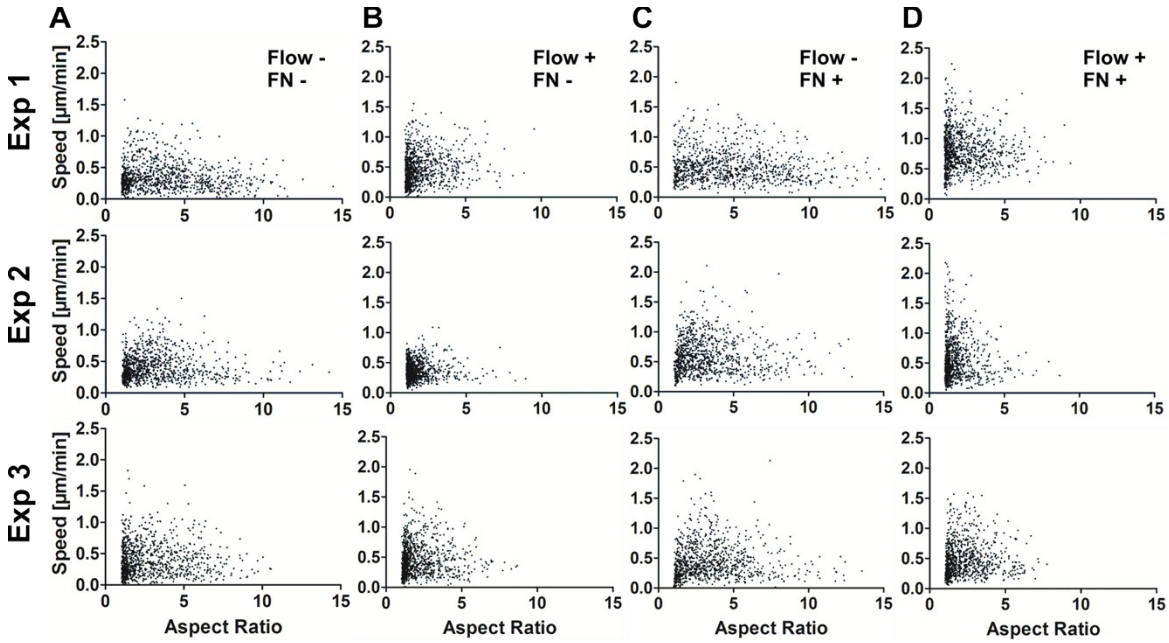

Figure S8: Scatter plots of hourly cell migration speed versus aspect ratio for three repeated independent experiments for four different flow and FN conditions.

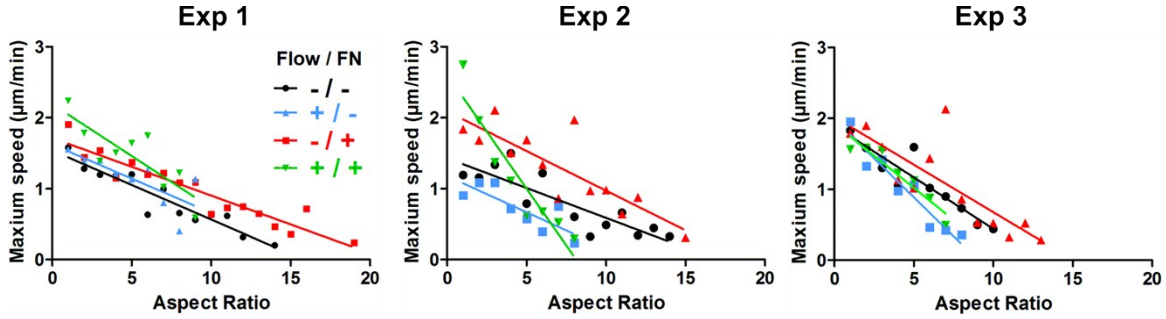

Figure S9: Maximum cell speed at a narrow range of aspect ratio is inversely correlated to the cell aspect ratio for three repeated independent experiments.

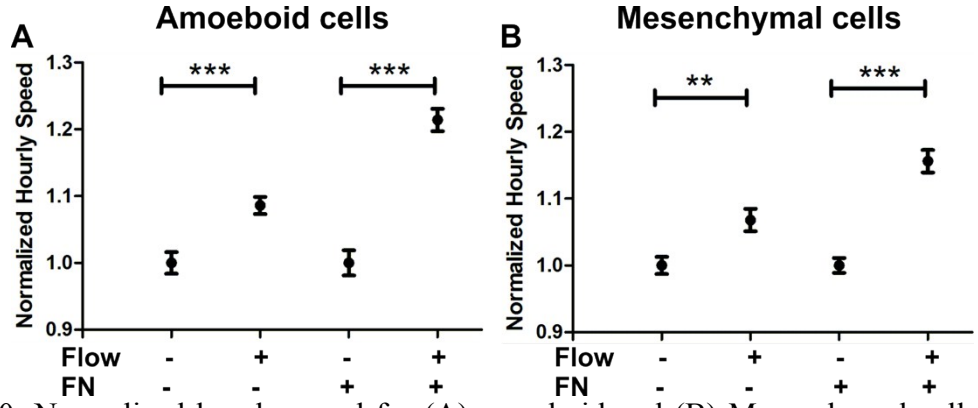

Figure S10: Normalized hourly speed for (A) amoeboid and (B) Mesenchymal cells indicating interstitial flows enhance the speed of both cell types.