Supplemental Materials

Molecular Biology of the Cell

Li et al.

Supplementary Figures

Figure S1: Microfluidic platform for applying interstitial flow (IF). (A) Detailed schematics (left) and the photograph (right) of the microfluidic system for interstitial flow study. To induce interstitial flow, two media reservoirs are bounded to the top of the microfluidic chamber. A media height difference can be established between these two reservoirs to maintain a hydrostatic pressure gradient. The media reservoirs are connected to the microchannels through the media ports. **(B)** To validate the interstitial flow velocity used in the microfluidic assay, time-lapse microscopy was used to track the movement of fluorescent micro-beads (200 nm) introduced into the media in the microfluidic system. Quantification of micro-bead movement shows that a media-height difference of 1.5 mm resulted in an IF velocity of 3.47 μm/s, similar to the velocity computed from Darcy's law model (red line; 3.34 μm/s). Moreover, no noticeable change in interstitial flow velocity was observed after 24 hrs of flow. For IF treatment longer than 24 hrs, the growth media height difference between the reservoirs was re-established every 24 hrs. Bars represent mean ± SEM of data (n=3, n=# of independent experiments; ns=not significant).

Figure S2: IF did not affect macrophage viability. Macrophages seeded in the microfluidic device were treated with 3 μm/s interstitial flow for 48 hrs, and the viability of the macrophages was assessed using live-dead assay. **(A)** Fluorescent micrographs showing the live/dead staining of macrophages treated with (right) or without interstitial flow (left) in the microfluidic devices (Green=live cell; Red=dead cell). **(B)** Quantification of the live-dead staining shows that interstitial flow treatment did not change the % live cell count compared to no-flow control. Bars represent mean ± SEM of data (n=3, ns=not significant).

Figure S3: Interstitial flow (IF) upregulated the expression of various M2 markers in macrophages similar to IL4 treatment. The expression levels of M2 markers in macrophages treated with IF (~3 μm/s, 48 hrs) were compared to that of macrophages treated with IL4 (20 ng/mL, 48 hrs). **(A-B)** Macrophages were treated with either IF or IL4 in the microfluidic devices, and the expression of M2 markers was assessed by immunofluorescence. Immunofluorescent images (left) and quantification (right) showing IF induced the expression of CD163 (A) and CD206 (B) in macrophages to levels similar to that of IL4 treatment. **(C-D)** Macrophages were treated with either IF or IL4 in the transwell flow chamber, and the expression of M2 markers was tested by western blot. Representative western blot images (top) and quantification (bottom) showing IF induced the expression of ArgI (C) and TGFβ (D) to levels similar to that of IL4 treatment. Bars represent mean ± SEM of data (fold change relative to no-flow/no-IL4 control).

Figure S4: Interstitial flow did not promote the activation of STAT1. Representative western blot images showing that IF did not promote the phosphorylation of STAT1, a transcription factor for M1 polarization, in macrophages compared to no flow control. In fact, no STAT1 phosphorylation was observed for both no-flow and flow conditions. Macrophages treated with 20 ng/mL mouse IL4 were used as negative controls for STAT1 activation. Meanwhile, macrophages that were treated with 20 ng/mL mouse IFNγ were used as a positive control for STAT1 phosphorylation.

Figure S5: Ruxolitinib treatment effectively inhibited STAT3 and STAT6 phosphorylation in macrophages. Macrophages were treated with interstitial flow for 30 min in the presence of ruxolitinib (Ruxo, 5 μM). Ruxolitinib treatment effectively inhibited the phosphorylation of STAT3 (left) and STAT6 (right) compared to DMSO controls.

Figure S6: Interstitial flow enhances the pro-invasive capabilities of macrophages. (A) Schematics of experiments designed to test the effects of IF on the abilities of macrophages to promote cancer cell migration and protrusion formation. Macrophages were first seeded in a collagen I ECM contained within the transwell flow chamber. Half of the macrophages were subsequently pre-treated with IF for 48 hrs in the chamber, while the other half were not subjected to IF (control macrophages). After the flow treatment,

conditioned media from the transwells were collected. MDA-MB-231 GFP breast cancer cells that were pre-seeded in a collagen I ECM in the microfluidic devices were treated with these conditioned media. The migration of cancer cells was tracked to quantify their movement. In a separate experiment, the transwell inserts containing macrophages were removed from the flow chamber after IF treatment and then placed into a six-well plate pre-seeded with MDA-MB-231 GFP breast cancer cells (MDA231), MDA-MB-435S melanoma cells (MDA435), or Du145 prostate cancer cells (Du145). The macrophages were co-cultured with cancer cells for an additional 24 hrs in the absence of flow to assess the effects of macrophages on cancer cell morphology. **(B)** Cancer cells (MDA231, MDA435, and Du145) co-cultured with macrophages pre-treated with IF have lower circularity than those co-cultured with control macrophages. Bars represent mean ± SEM of data from 50-100 cells (n=3; **: $p<0.01$, ***: $p<0.001$).

Figure S7: Inhibiting TGFβ in conditioned media collected from IF-treated macrophages reduced its ability to enhance cancer cell migration. Representative cancer cell migration trajectory of MDA231 cells treated with conditioned media collected from macrophages pre-treated with IF (flow). Inhibiting TGFβ in the conditioned media using anti-TGFβ antibodies (10 μg/mL) reduced cancer cell migration compared to IgG control (10 μg/mL). Quantification is shown in Figure 4.

Figure S8: Interstitial flow (IF) promotes M2 migration characteristic. Migration characteristics of IF-treated macrophages were compared to those of macrophages treated with LPS (to chemically induce an M1 phenotype) or IL4 (to chemically induce an M2 phenotype). **(A)** Representative migration trajectories for macrophages cultured in ECM under no-flow (top left), flow (top right), LPS (bottom left), and IL4 (bottom right) treatment conditions. Note that no IF was applied to macrophages subjected to LPS or IL4 treatment, and no chemical treatment was applied to macrophages subjected to IF. **(B)** Quantification of macrophage migration total speed (up) and directedness (down) showing that IL4 treatment of macrophages resulted in higher migration total speed and directedness compared to LPS treatment. In addition, macrophages treated with IF have similar migration total speed and directedness compared to that of macrophages treated with IL4, but not macrophages treated with LPS. Bars represent mean ± standard error of mean (SEM) of data from 40-100 cells (n=3).

Figure S9: β1 integrin plays a key role in IF-induced Akt and FAK activation. Macrophages were treated with anti-β1 integrin blocking antibody (15 μ g/mL) and subsequently subjected to IF treatment for 15 min. Representative western blot images showing that inhibiting β1 integrin resulted in reductions in flow-enhanced phosphorylation of Akt at Ser473 (A) and FAK at Tyr397 (B) compared to IgG isotype control.

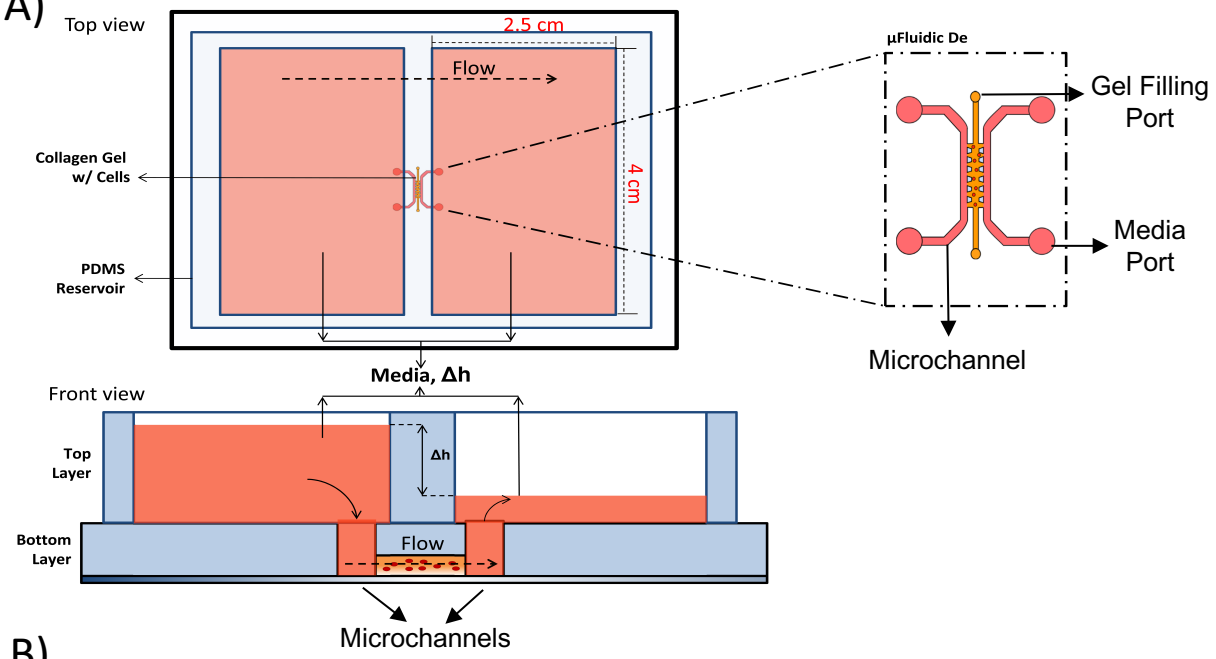
Figure S10: IF enhanced the percentage of macrophages migrating upstream (against the direction of flow). Quantification of the percentage of the Raw macrophages and bone marrow-derived macrophages (BMDM) that migrated upstream and downstream. More macrophages migrated upstream under IF treatment for both Raw macrophages (A) and BMDM (B). In contrast, roughly equal numbers of BMDM and Raw macrophages migrated in both directions for no flow conditions. Bars represent mean ± SEM of data from 3 independent experiments.

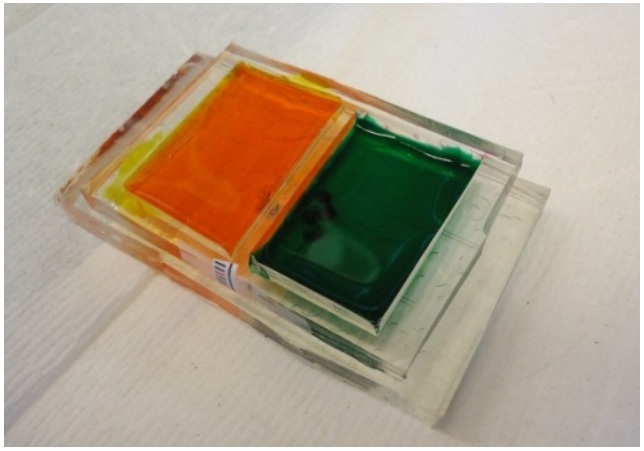
Figure S11: Definitions of forward migration index parallel ($FMI_{//}$) or perpendicular ($FMI_{\perp}$) to interstitial flow direction. $D_{//}$ is displacement of cell against the flow direction, $D_{\perp}$ is displacement of cell

perpendicular to the flow direction, L is total distance the cell travelled. $FMI_{//}=D_{//}/L$, which represents how persistently the cell travels against the flow (+ upstream, - downstream, 0 no preference). $FMI\perp= D\perp/L$, which represents how persistently the cell travels perpendicular to the flow direction.

Figure S12: Interstitial flow promotes the accumulation of actin and protrusion formation against the direction of flow. (A) Actin distribution in macrophages was quantified with actin distribution score (ADS), which is defined as the difference between the average fluorescent intensities of actin on the upstream ($<I_{up}>$) and downstream side of the cell ($<I_{down}>$) divided by the average fluorescent intensity of the entire cell. A positive ADS indicates actin is accumulated on the upstream side, while a negative ADS indicates actin is accumulated on the downstream side. A zero ADS indicates no spatial preference. **(B)** Interstitial flow treatment resulted in a positive population-average ADS, indicating that actin accumulated to the upstream side in most macrophages treated with IF. This is in contrast to macrophages that were not treated with flow, which showed a population-average ADS of zero. **(C)** The spatial distribution of macrophage protrusion was quantified by protrusion distribution score (PDS), which is defined as the difference between the perimeters of upstream side (P_{up}) and downstream side (P_{down}) of the cells divided by the average. A positive PDS means the protrusion preferentially formed on the upstream side, while a negative PDS means protrusion formed on the downstream side. A zero PDS indicates no spatial preference. **(D)** IF treatment resulted in a positive population average PDS, meaning IF drives the formation of macrophage protrusion against the flow direction. Bars represent mean ± SEM of data from 40-80 cells (n=3; **: $p<0.01$).

A)


Top view
2.5 cm
Flow
Collagen Gel
w/ Cells
4 cm
PDMS
Reservoir
µFluidic De
Gel Filling
Port
Media
Port
Microchannel
Media, Δh
Front view
Top
Layer
Δh
Bottom
Layer
Flow
Microchannels



B)

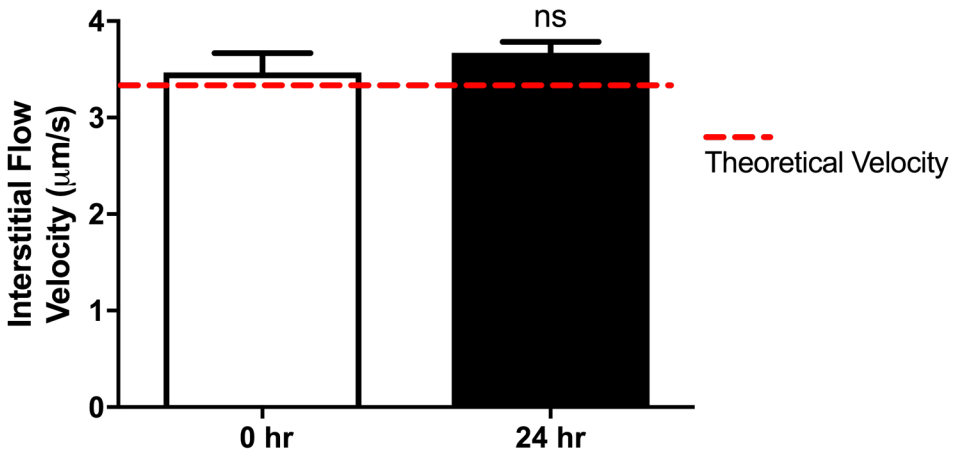

ns
4
3
2
1
0
Interstitial Flow
Velocity (µm/s)
Theoretical Velocity
0 hr
24 hr

Fig. S1

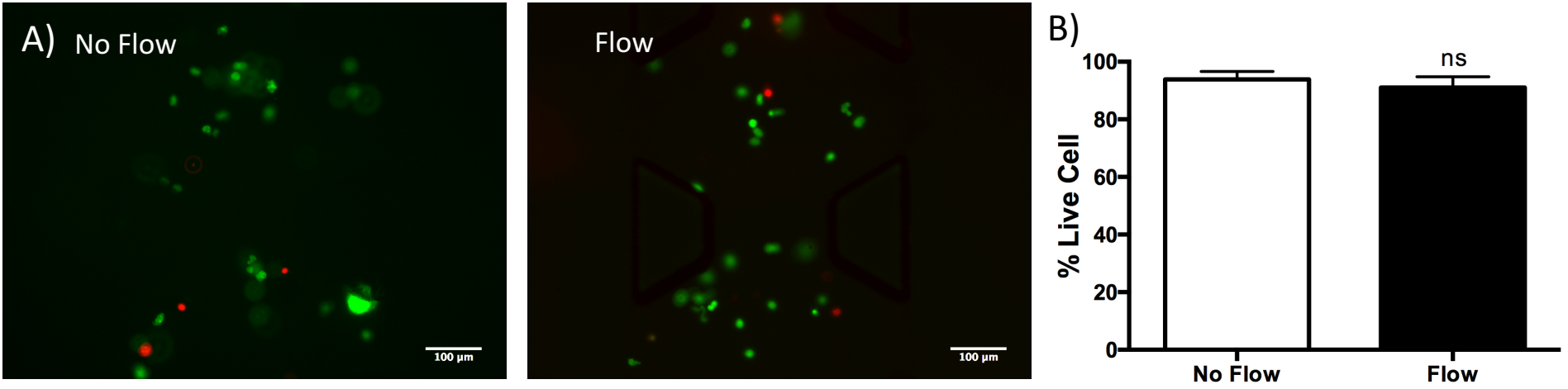

A)
No Flow
100 µm
Flow
100 µm
B)
ns
% Live Cell
100
80
60
40
20
0
No Flow
Flow

Fig. S2

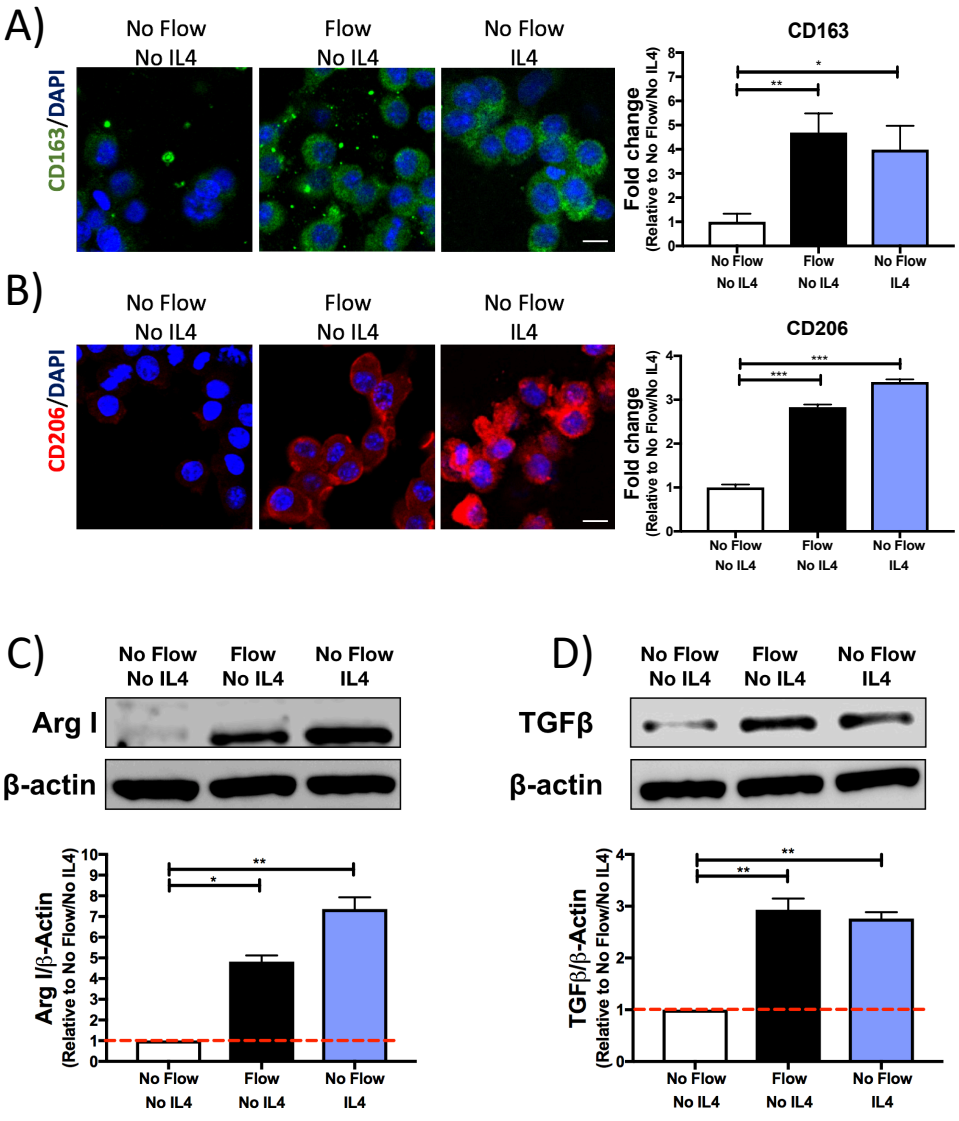

A)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
CD163/DAPI
CD163
Fold change
(Relative to No Flow/No IL4)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
B)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
CD206/DAPI
CD206
Fold change
(Relative to No Flow/No IL4)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
C)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
Arg I
β-actin
Arg I/β-Actin
(Relative to No Flow/No IL4)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
D)
No Flow
No IL4
Flow
No IL4
No Flow
IL4
TGFβ
β-actin
TGFβ/β-Actin
(Relative to No Flow/No IL4)
No Flow
No IL4
Flow
No IL4
No Flow
IL4

Fig. S3

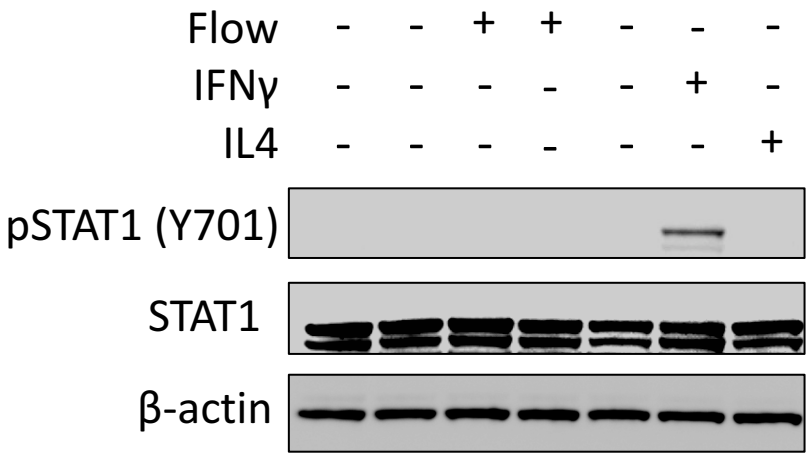


Fig. S4

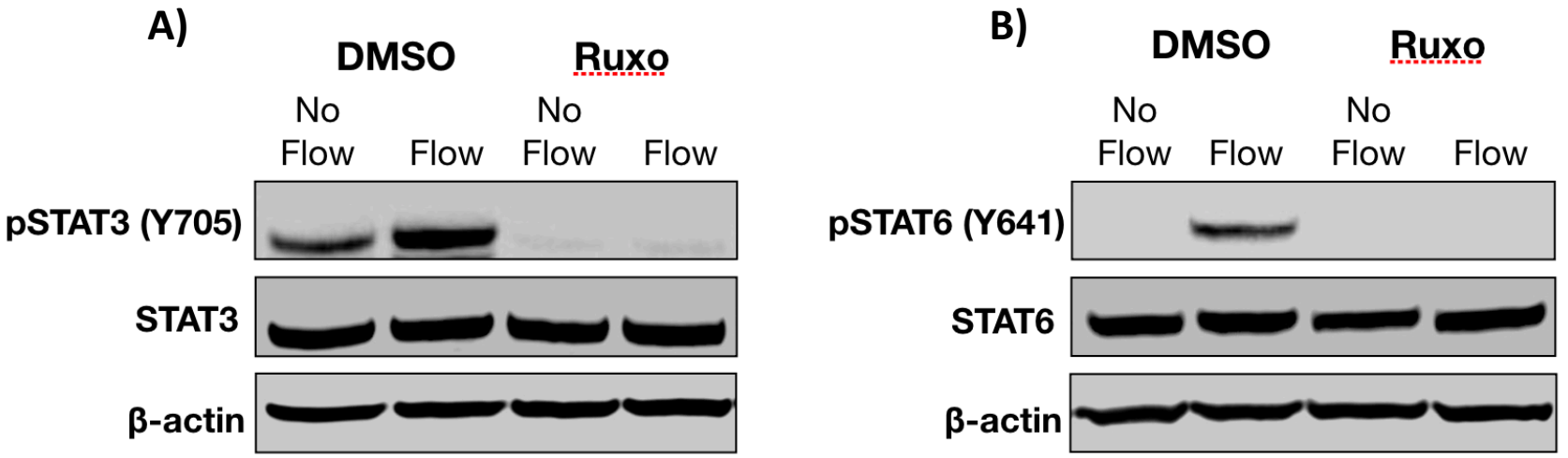

A)
DMSO
Ruxo
No Flow
Flow
No Flow
Flow
pSTAT3 (Y705)
STAT3
β-actin
B)
DMSO
Ruxo
No Flow
Flow
No Flow
Flow
pSTAT6 (Y641)
STAT6
β-actin

Fig. S5

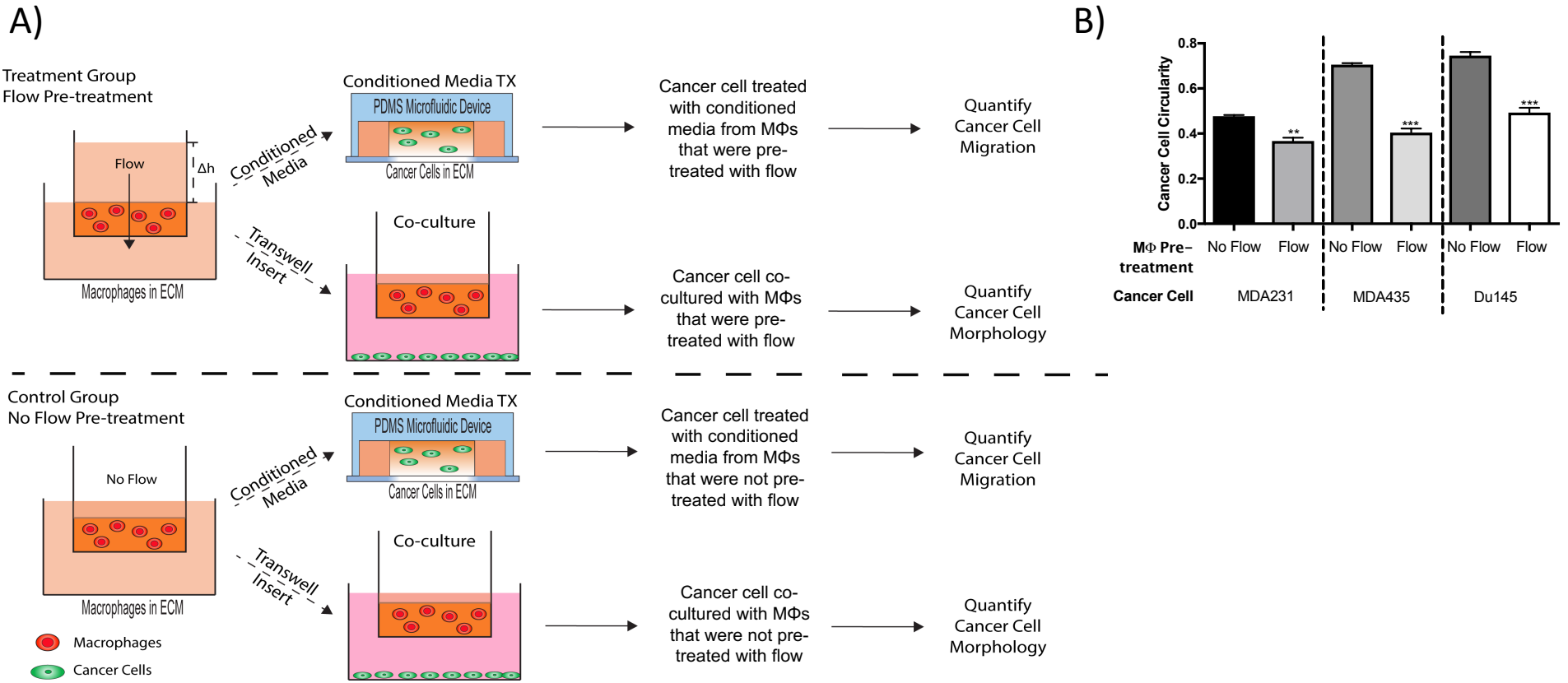
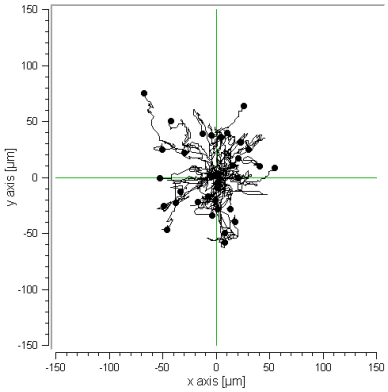

A)
Treatment Group
Flow Pre-treatment
Flow
Δh
Macrophages in ECM
Conditioned Media
Conditioned Media TX
PDMS Microfluidic Device
Cancer Cells in ECM
Transwell Insert
Co-culture
Cancer cell treated with conditioned media from MΦs that were pre-treated with flow
Quantify Cancer Cell Migration
Cancer cell co-cultured with MΦs that were pre-treated with flow
Quantify Cancer Cell Morphology
Control Group
No Flow Pre-treatment
No Flow
Macrophages in ECM
Conditioned Media
Conditioned Media TX
PDMS Microfluidic Device
Cancer Cells in ECM
Transwell Insert
Co-culture
Cancer cell treated with conditioned media from MΦs that were not pre-treated with flow
Quantify Cancer Cell Migration
Cancer cell co-cultured with MΦs that were not pre-treated with flow
Quantify Cancer Cell Morphology
Macrophages
Cancer Cells
B)
Cancer Cell Circularity
0.8
0.6
0.4
0.2
0.0
**
***
***
***
MΦ Pre-treatment
No Flow
Flow
No Flow
Flow
No Flow
Flow
Cancer Cell
MDA231
MDA435
Du145

Fig. S6

Cancer Cell Migration Trajectory

MΦ Pre-treatment

Flow + IgG



Flow + a-TGFβ

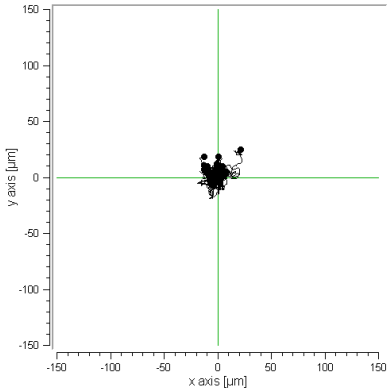
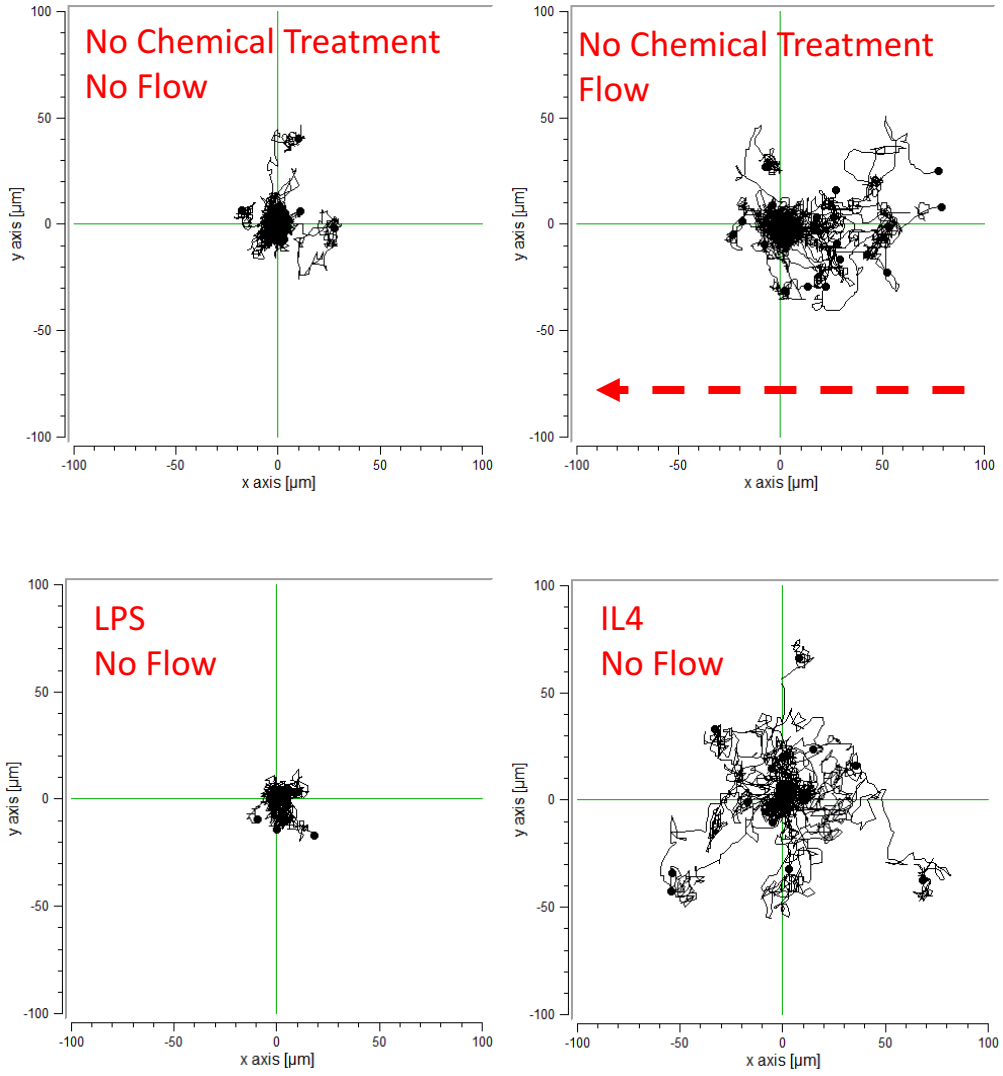


Fig. S7

A) Macrophage (MΦ) Migration Trajectory



B)

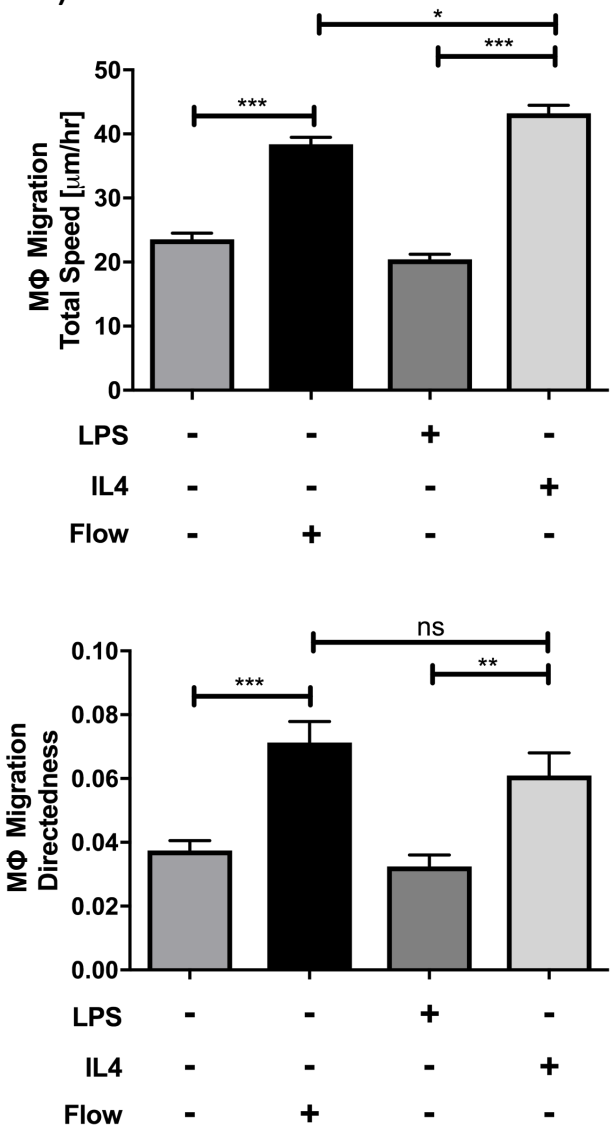
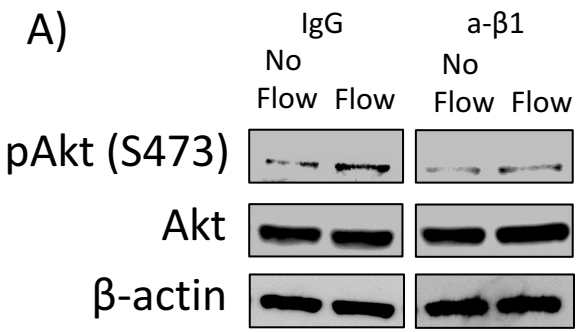


Fig. S8


A)
IgG
a-β1
No Flow
Flow
No Flow
Flow
pAkt (S473)
Akt
β-actin

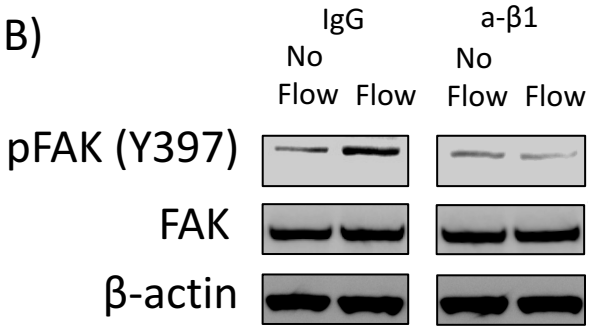
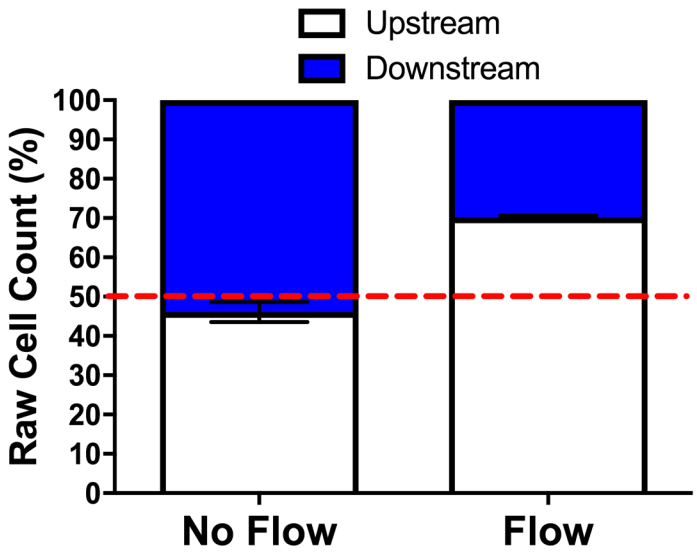

B)
IgG
a-β1
No Flow
Flow
No Flow
Flow
pFAK (Y397)
FAK
β-actin

Fig. S9

A)


Upstream
Downstream
Raw Cell Count (%)
100
90
80
70
60
50
40
30
20
10
0
No Flow
Flow

B)

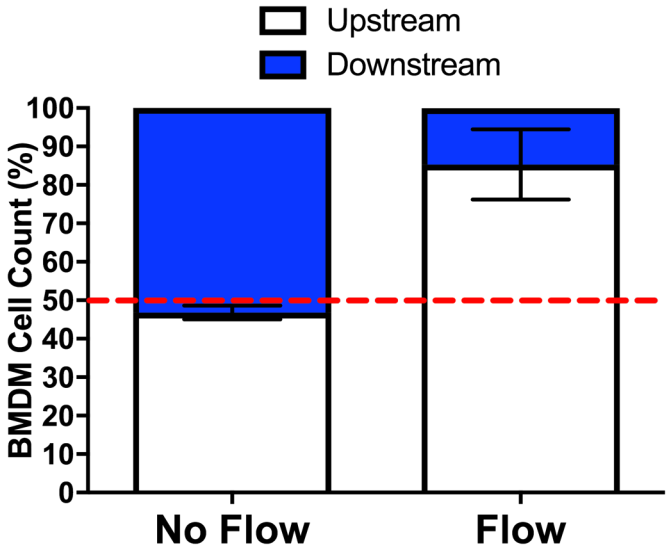

Upstream
Downstream
BMDM Cell Count (%)
100
90
80
70
60
50
40
30
20
10
0
No Flow
Flow

Fig. S10

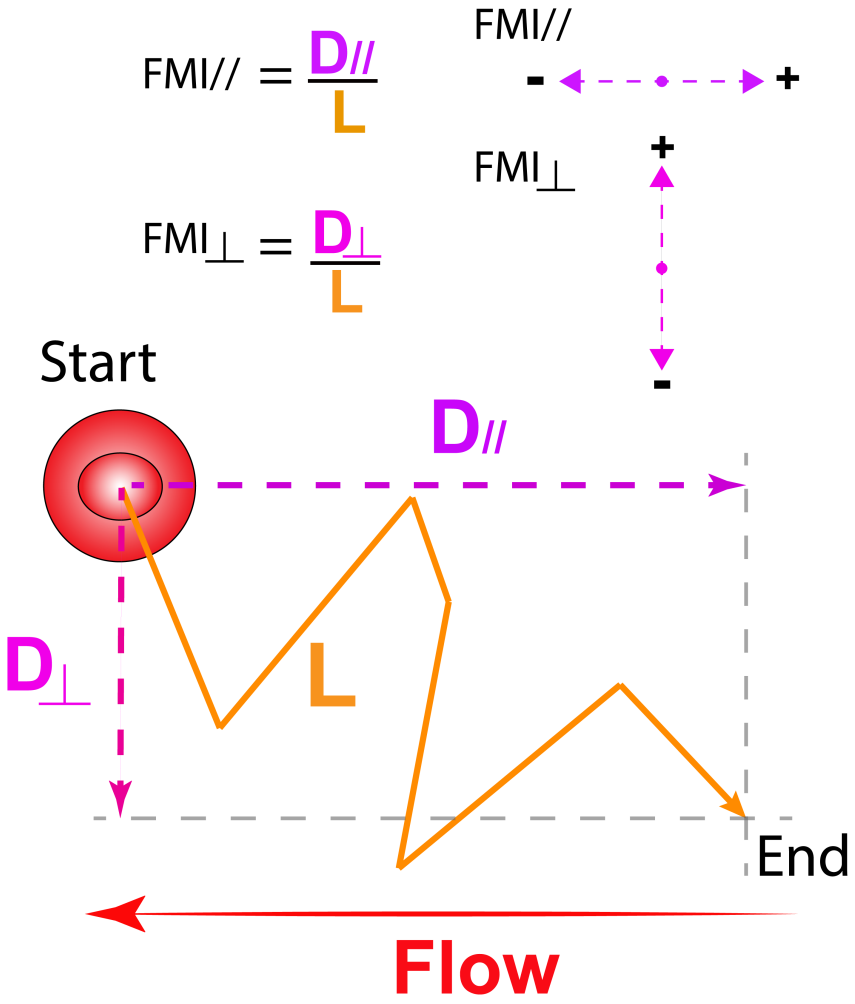


Fig. S11

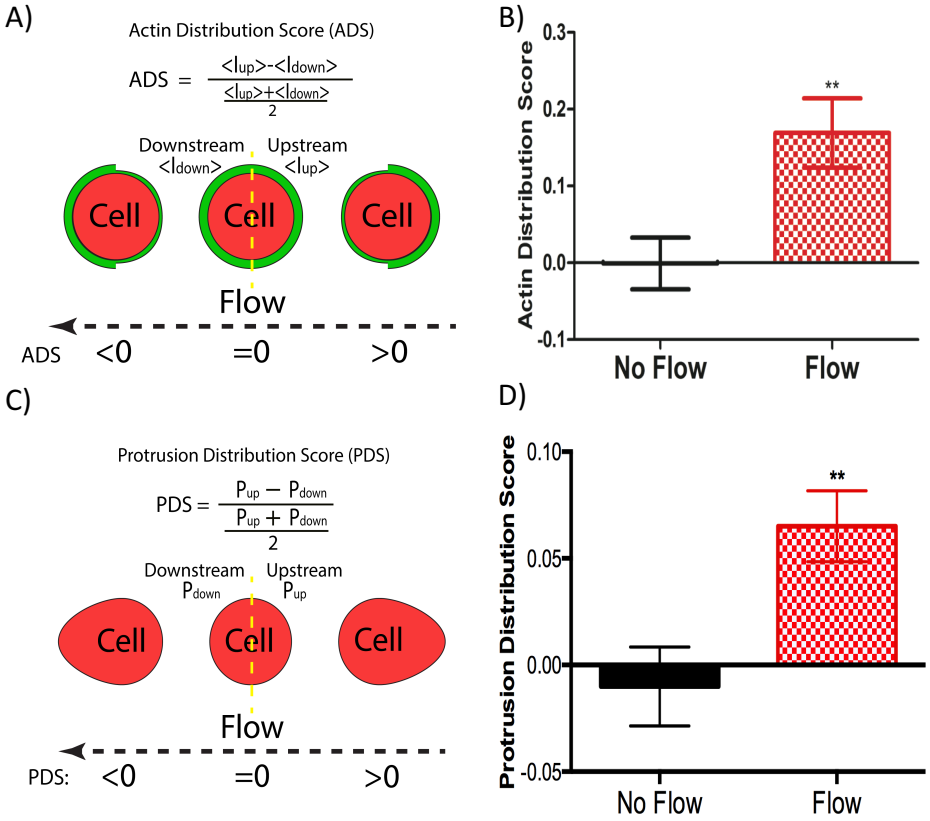

A)
Actin Distribution Score (ADS)
ADS = <Iup>-<Idown> / <Iup>+<Idown> / 2
Downstream
<Idown>
Upstream
<Iup>
Cell
Cell
Cell
Flow
ADS
<0
=0
>0
B)
Actin Distribution Score
0.3
0.2
0.1
0.0
-0.1
**
No Flow
Flow
C)
Protrusion Distribution Score (PDS)
PDS = Pup − Pdown / Pup + Pdown / 2
Downstream
Pdown
Upstream
Pup
Cell
Cell
Cell
Flow
PDS:
<0
=0
>0
D)
Protrusion Distribution Score
0.10
0.05
0.00
-0.05
**
No Flow
Flow

Fig. S12