Supplementary data for

Fluid Shear Stress in a Logarithmic Microfluidic Device Enhances Cancer Cell Stemness Marker Expression

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1. Cell seeding protocol

For each experiment the same batch of cells was used. To validate the reproducibility of the results different batches of cells were revived and introduced in the experiments. Cells were seeded in the device at a concentration of 6 to 7 million cells per ml in each chamber. This resulted in a nearly confluent monolayer of cells in the device. The volume of each chamber is 18 μl (0.1 mm × 6 mm × 30 mm) which allowed 1.08×10^5 to 1.26×10^5 cells to be seeded in each chamber. For static culture a glass plate was bonded with a PDMS slab with rectangular holes in it. The holes have a crossectional area same as that of a single culture chamber in the device (1.8 cm^2). After bonding it generated slots with the same crossectional area as that of the cell culture chamber in the microfluidic device with a glass bottom. Now this bonded structure was processed the same way as the microfluidic device to generate an APTES and collagen coating similar to that of the microfluidic device. After the coating, it was used for static culture by seeding from the same cell suspension with the same volume as that of a single culture chamber.

2. Macro for cell counting

For counting number of cells in a fluorescent image custom macro was developed in imageJ software. It counted the number of nuclei stained in Hoechst dye (blue). Fluorescent images were converted to mask which converts blue nucleus to black particles. The numbers of particles were then count with particle counting tool. The text in the macro and representative images are given below.

```
run("8-bit");

run("Bandpass Filter...", "filter_large=40 filter_small=3 suppress=None tolerance=5 autoscale saturate");

setAutoThreshold("Default");

//run("Threshold...");

//setThreshold(0, 93);

run("Convert to Mask");

run("Convert to Mask");

run("Watershed");

run("Analyze Particles...", "size=350-Infinity show=Outlines exclude clear summarize");
```
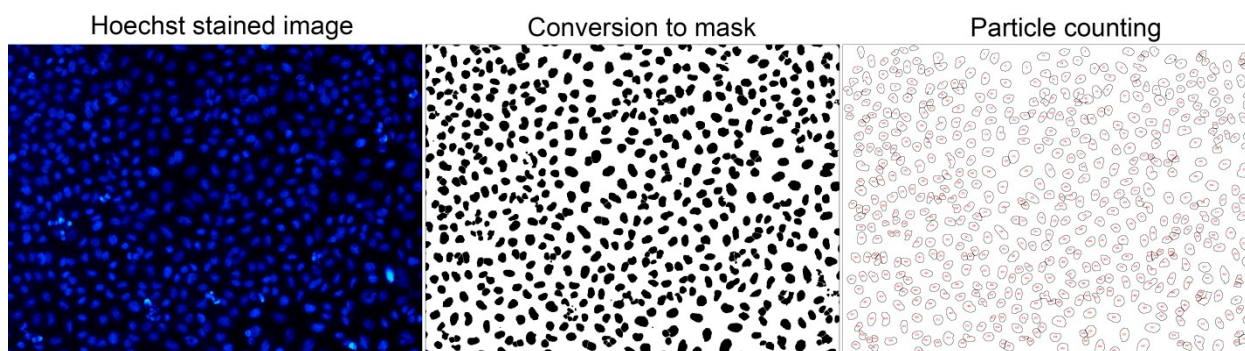

Figure S1: Steps for counting cell number using imageJ macro. Blue fluorescence represents nucleus stained with Hoechst.

3. Macro for EdU positive cells

For counting EdU positive cells another macro was developed in imageJ to count the number of cells showing EdU fluorescence. Fluorescent images were converted to mask which converts orange stained nucleus to black particles. The numbers of particles were then count with particle counting tool. The detail text of the macro is given below. The representative images are followed.

```
run("8-bit");

run("Bandpass Filter...", "filter_large=40 filter_small=3 suppress=None tolerance=5 autoscale saturate");

setAutoThreshold("Intermodes");

//run("Threshold...");

setThreshold(0, 125);

//setThreshold(0, 125);

setOption("BlackBackground", false);

run("Convert to Mask");

run("Convert to Mask");

run("Watershed");

run("Analyze Particles...", "size=350-Infinity show=Outlines exclude clear summarize");
```
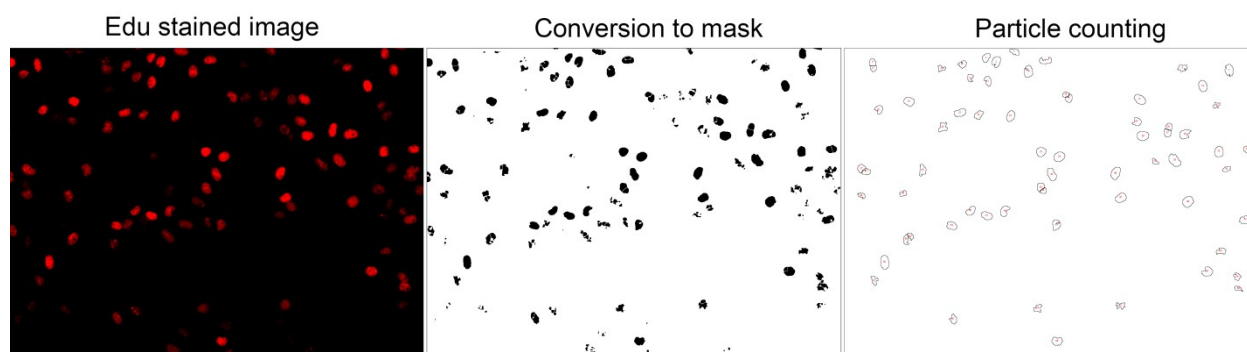

Figure S2: Steps for EdU positive cell counting using imageJ macro. Orange fluorescence represents nucleus conjugated with EdU followed by Alexa fluor 555 staining.

4. Colony forming assay protocol

Post FSS treatment, samples were seeded (600 cells per well for FSS treatment and 3000 cells per well for FSS+DOX treatment) in a 6 well plate as a single cell suspension and grown for 10 days in DMEM HG media with 10% FBS and other supplements as mentioned earlier. After washing the cells, the colonies were fixed with ice cold methanol for 30 min. Later, they were stained using 0.5% crystal violet in 25% methanol (w/v) solution for 30 min. This was followed by a wash with sterile water. The plates were air-dried and the colonies were imaged using smartphone camera keeping the distance between camera and 6 well plate same for all the samples. The images were processed in imageJ free software for counting of the number of colonies. Firstly, the image was cropped to a circle encompassing all the colonies. Then, the image was converted to 8 bit and a Fast Fourier Transformation with default setting was followed. This was done to generate a proper demarcation between the colonies and background. Then the threshold was adjusted to make the colonies black and the background white. Now the number of black particles were counted using the 'analyze particles' tool. Particles more than 10 pixels and less than 500 pixels were treated as colonies. The number of colonies was normalized with the static group for each sample.
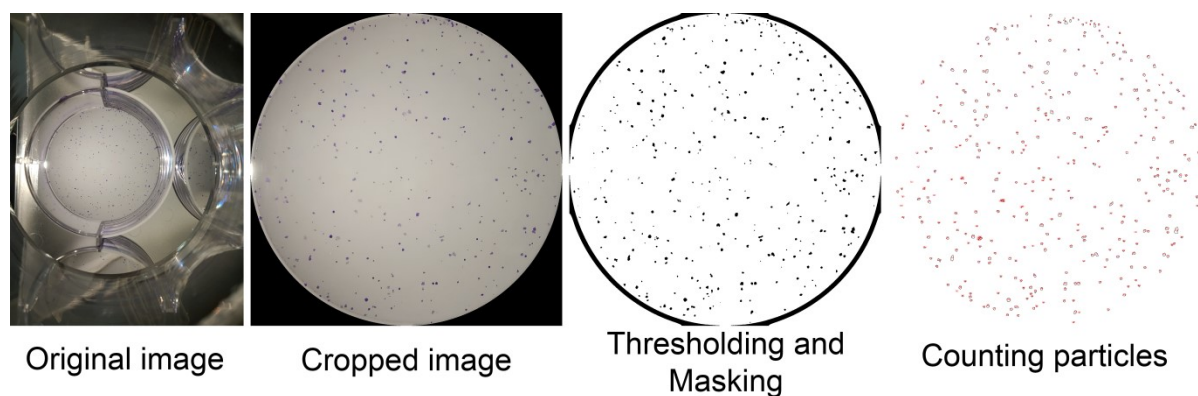

Original image   Cropped image   Thresholding and Masking   Counting particles

Figure S3: Procedure for counting number of colonies in crystal violet stained images. Original image taken in mobile camera is modified in imageJ to complete the count.
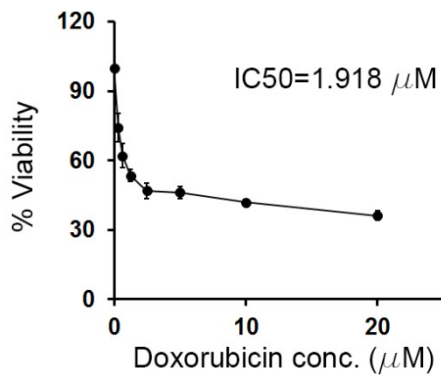
5. MTT assay IC50 calculations

Figure S4: IC50 calculation for doxorubicin in HeLa through MTT assay.

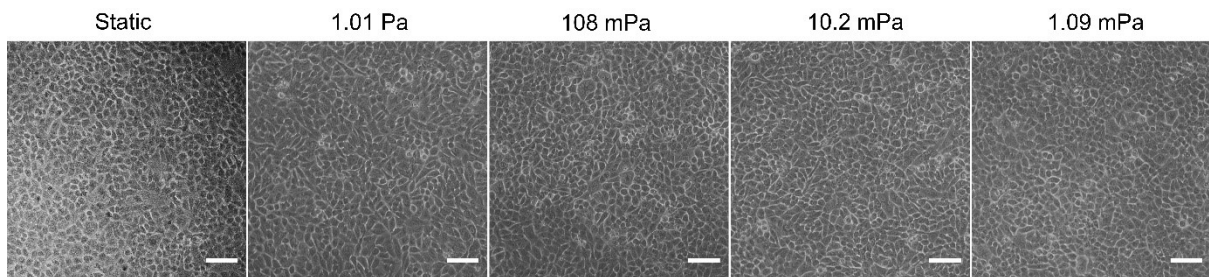## 6. Cell morphology after flow treatment

Figure S5: Cell morphology after 12 hrs of continuous flow. No morphological difference was seen among the groups. Scale bar = 100 μm.
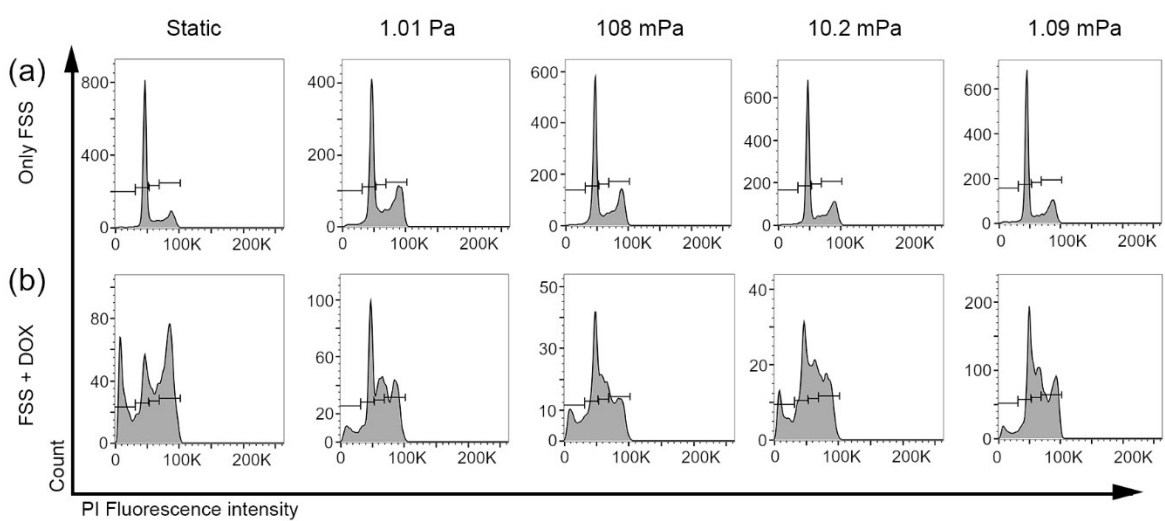
7. Cell cycle analysis

Figure S6: Cell cycle phases in different samples. (a) Only FSS treatment, (b) FSS+ DOX treatment.

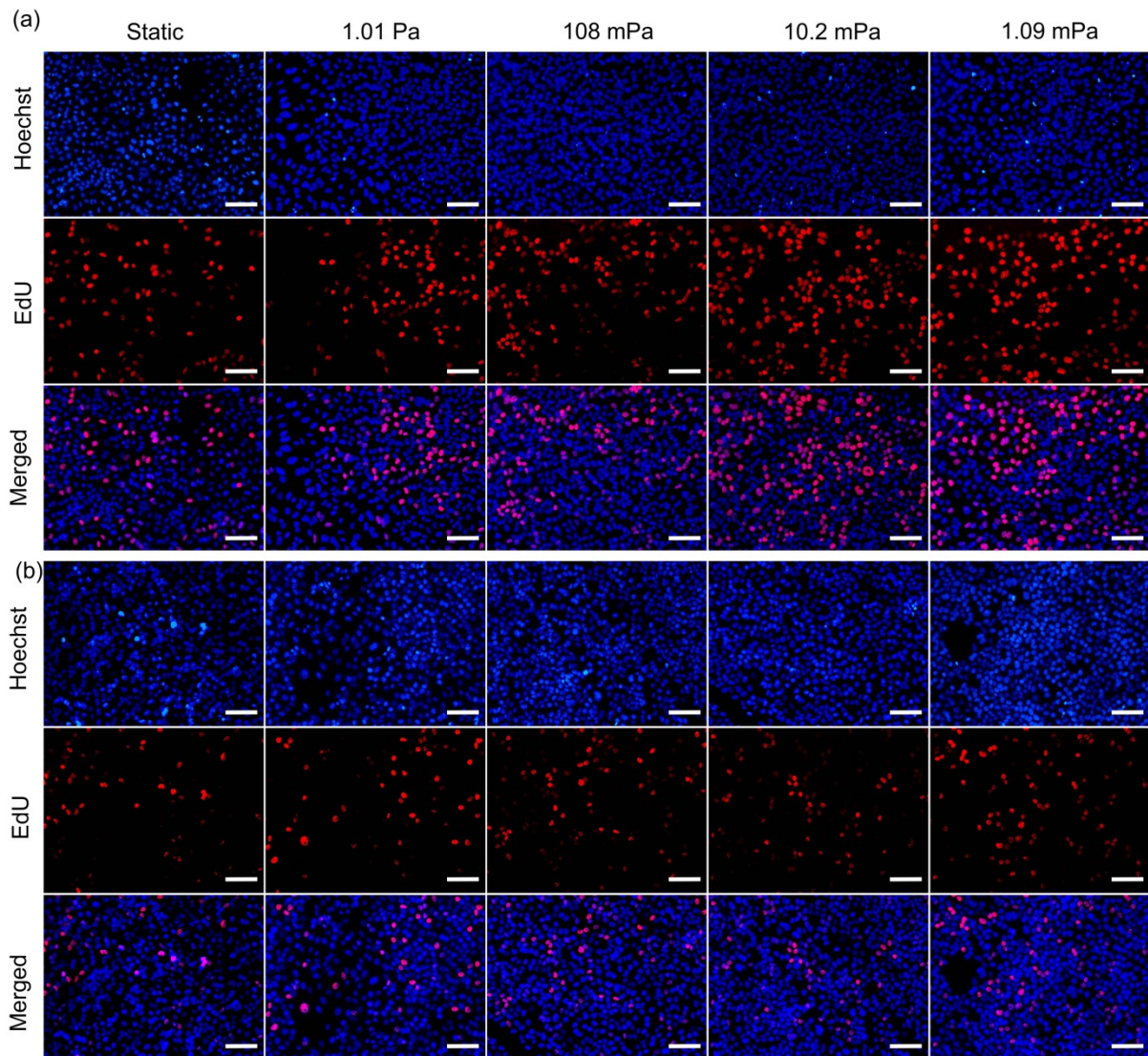## 8. Cell proliferation analysis

Figure S7: Fluorescent images for Hoechst (blue) and Edu (orange stains. (a) Only FSS treatment, (b) FSS+DOX treatment. Scale bar =100 μm.

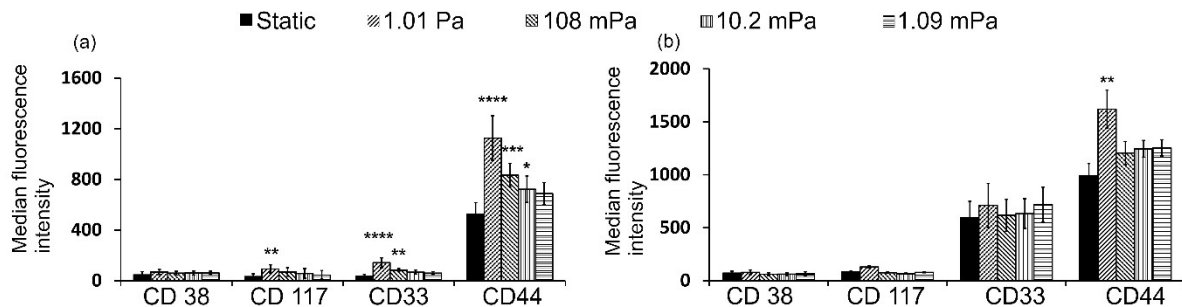## 9. Cell surface marker expression analysis

Figure S8: CD Marker expression shown in median intensity of the fluorescent signal. (a) only FSS, (b) FSS +DOX, CD33 and CD44 expression became approximately 6 and 2 times after introduction of doxorubicin.

10. Tables

Table S1: List of primers used for RT-pcr

Beta actin forward	TCATGAAGATCCTCACCGAG	190 bp
Beta actin reverse	TTGCCAATGGTGATGACCTG	
N-cadherin forward	CCCTGCTTCAGGCGTCTGTA	52 bp
N-cadherin reverse	TGCTTGCATAATGCGATTTCACC	
Sox2 forward	GCGGAAAACCAAGACGCTC	150 bp
Sox2 reverse	ATGTGCGCGTAACTGTCCAT	
Vimentin forward	CCACCAGGTCCGTGTCCTCGT	123 bp
Vimentin reverse	CGCTGCCCAGGCTGTAGGTG	