Electronic Supplementary Material (ESI) for Lab on a Chip.

A Cell-Based Sensor of Fluid Shear Stress for Microfluidics

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Supplemental Information

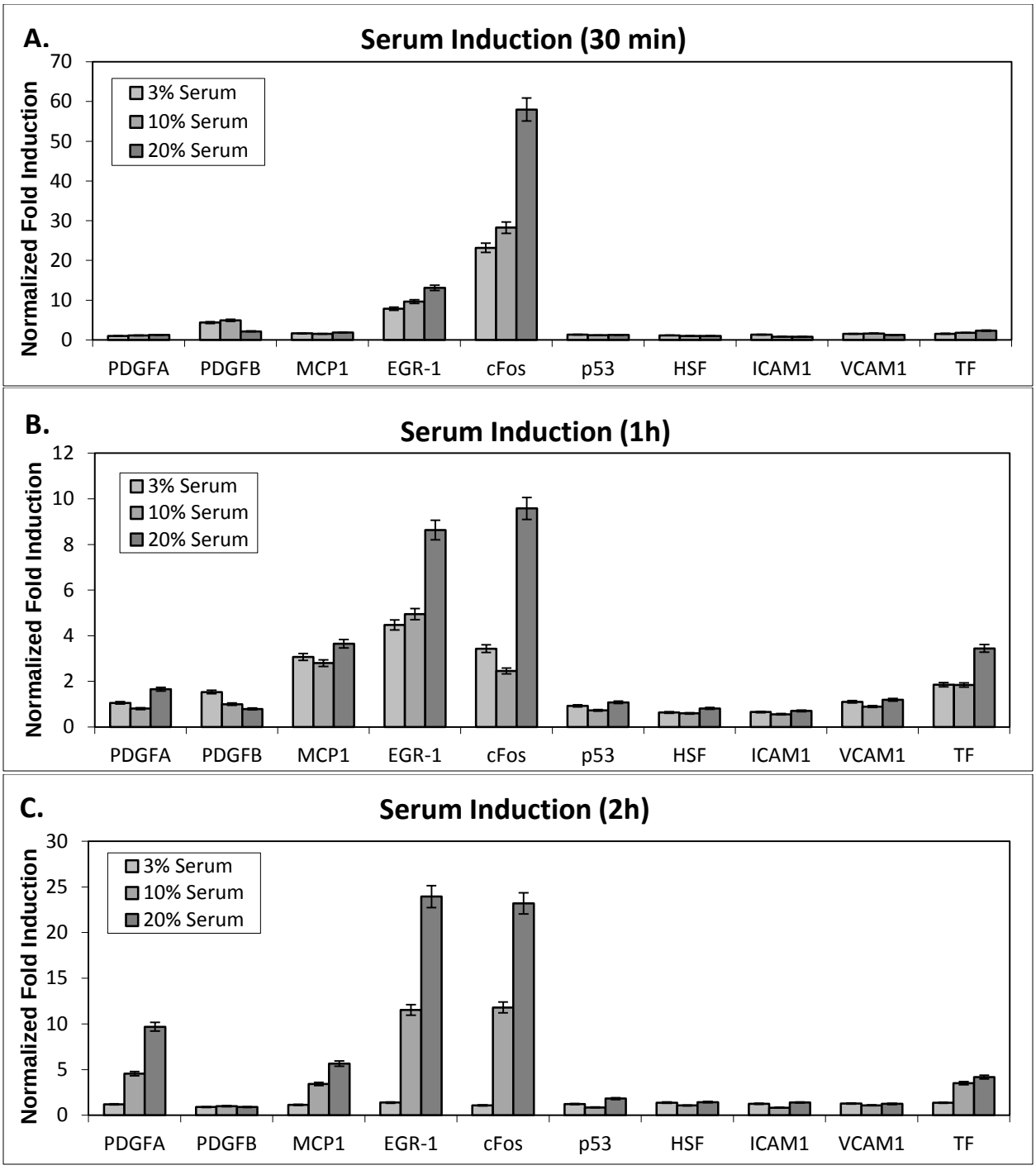
PCR Primers

The following table lists the PCR Primers used for qRT-PCR studies.

Gene	GeneBank ID	Primer	Sequence (5'-->3')
p53	NM_001127233.1	Fwd	AGGGCTCACTCCAGCCTCCAG
		Rev	AGGGGAGGGATGAAGTGATGGG
HSF1	NM_008296.2	Fwd	TGCTGGAGCCCGAGTGGGAA
		Rev	TGCCGCACGAAGCTAGCCAT
PDGFB	NM_011057.3	Fwd	ATCCAGGGAGCAGCGAGCCAA
		Rev	CCGCCTTGTCATGGGTGTGCT
MCP-1	NM_011333	Fwd	CAGCCAGATGCAGTTAACGCCC
		Rev	ACCTGCTGCTGGTGATCCTCTTGT
PDGFA	NM_008808	Fwd	ACTCCGTAGGGGCTGAGGATGC
		Rev	CGAATGGGCACAGGCCGCTT
TF	NM_010171	Fwd	GCGGGTGCAGGCATTCCAGAG
		Rev	TAGTTGGTGGGTTTGGGTTGCCA
c-Fos	NM_010234.2	Fwd	GGCTTACGCCAGAGCGGGAA
		Rev	GGAGATAGCTGCTCTACTTTGCCCC
EGR-1	NM_007913.5	Fwd	GCACCTGACCACAGAGTCCTTTTC
		Rev	GGTGATGGGAGGCAACCGAG
ICAM-1	NM_010493.2	Fwd	ACGTGCTGTATGGTCCTCGGCT
		Rev	AGGAGATGGGTTCCCCCAGGC
GAPDH	NM_008084.2	Fwd	CACTGAGCATCTCCCTCACA
		Rev	GTGGGTGCAGCGAACTTTAT

Serum inductions

Cells were seeded in 6-well plates to reach 80% confluence. Serum free media (0.15% serum) was introduced to cell cultures for 24 hours in order to serum-starve cells prior to induction. The serum starved cells were then incubated with regular cell culture media with 3, 10 and 20% (v/v) serum concentrations for 30 minutes, 1 hour and 2 hour durations. The reference controls were cell cultures with no serum inductions. Cells were lysed for RNA extraction immediately after the serum exposure. Induced expression was normalized to basal expression of serum starved cells. The results are shown in SI Figure 1.



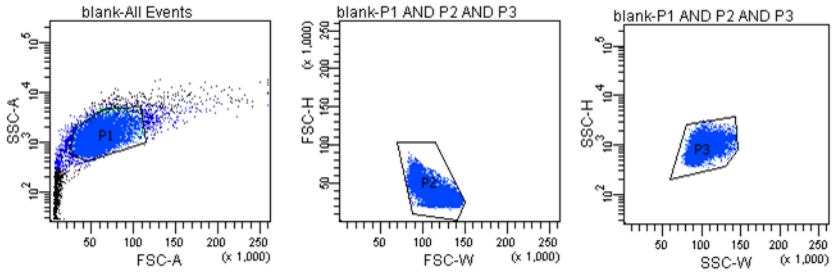
SI Figure 1. GAPDH-normalized gene expression of serum-induced cells at 30 min (A), 1 hour (B) and 2 hour (C) exposure duration. N = 2 experiments, error bar: standard error of mean.

Flow Cytometry Gating and Setup

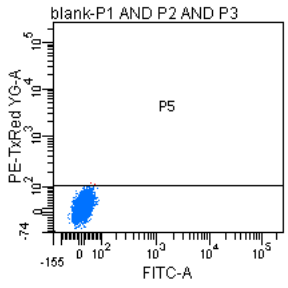
For the detection channels, PE-TexasRed-YG-A (red channel) filter was used with an excitation wavelength of 561 nm and emission detector centered at 610 nm with a 20 nm bandwidth. The reference channel was chosen to be FITC with an excitation wavelength of 488 nm and its emission detector centered at 530 nm with a 30 nm bandwidth. The flow cytometry experimental template was setup using untransfected cells, stable RFP expressing cells (positive control) and stable YPet expressing cells (secondary reference positive control).

Flow cytometry instrument gains and settings were set in order to capture the dynamic range of expression based on these controls and were used consistently among all flow cytometry experiments. The primary gates first were set to select the majority of cell populations from the forward and side scatter information. The subset of all the events analyzed that qualified as cells were set by the intersection gates named P1, P2, and P3 within the forward and side scatter channels as shown in SI Figure 2A . The combined population from the intersection of P1, P2 and P3 gates was analyzed for expression of red fluorescence against FITC reference channel. The fluorescence cluster of the untransfected (blank) cell population was centered with an approximate zero mean fluorescence in all channels. A horizontal gate was set above to threshold the maximum background fluorescence from the control population to create a sub-population called P5. The distribution fluorescence intensity was analyzed by a histogram of the red channel. An example of P5 within P1, P2 and P3 gates, as well as the RFP intensity distribution histogram for a non-fluorescent cell population is shown in SI Figure 2B, C. The percentage of cells in the P5 gate was termed '% activated cells' for all the experimental conditions. When comparing the fold induction of percent activated population, or in other words, the shift in the fluorescence distribution histogram, the % activated cells in P5 of the induced population was normalized by that in non-induced population. To analyze the change in the fluorescence upon induction, the mean red intensity of the combined P1, P2 and P3 population was compared from before and after induction. The mean RFP fluorescence of this population after induction was divided by that of the uninduced population and was termed as the normalized fold RFP induction.

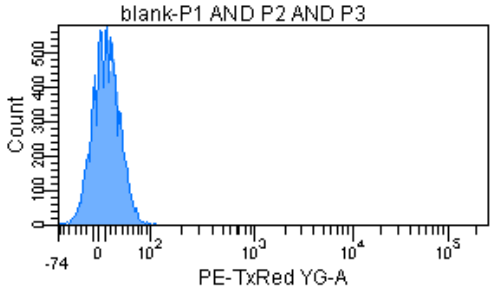
A.



B

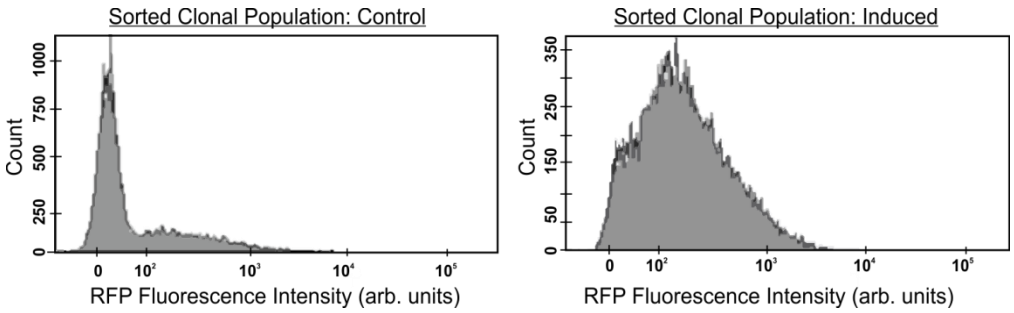


C.



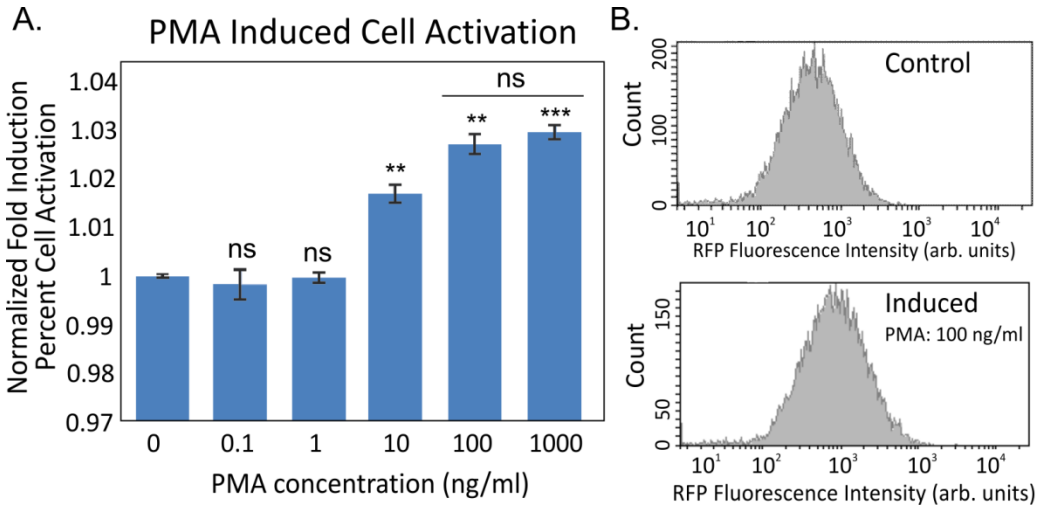
SI Figure 2. A. Forward and side scatter gates chosen in flow cytometry to highlight regions of live cells. **B.** Gating the auto-fluorescence of blank cells to create P5 region. **C.** RFP intensity distribution plotted for the entire cell population within the side and forward gates (P1, P2, and P3) in the PE-TexasRed (RFP) channel.

Sorted Clonal cells were induced with PMA and the best clone was selected on the criteria of best induction at the population level. SI Figure 3 shows the RFP intensity histograms for the final chosen clone before and after PMA exposure.



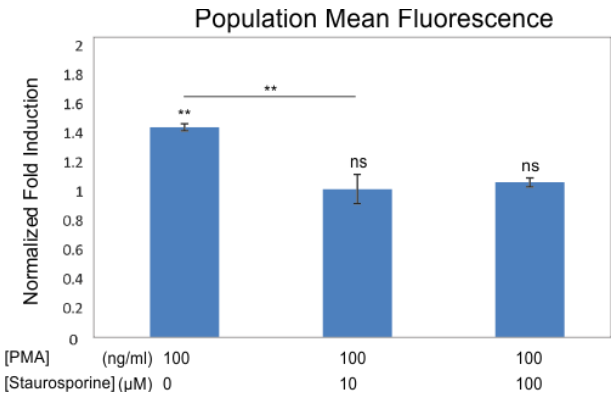
SI Figure 3. Population RFP intensity histograms for control and induced conditions for the clonal population

The fraction of induced cells expressing RFP levels above the threshold of maximum fluorescence level of the control population were compared against PMA doses, and are shown in SI Figure 4.

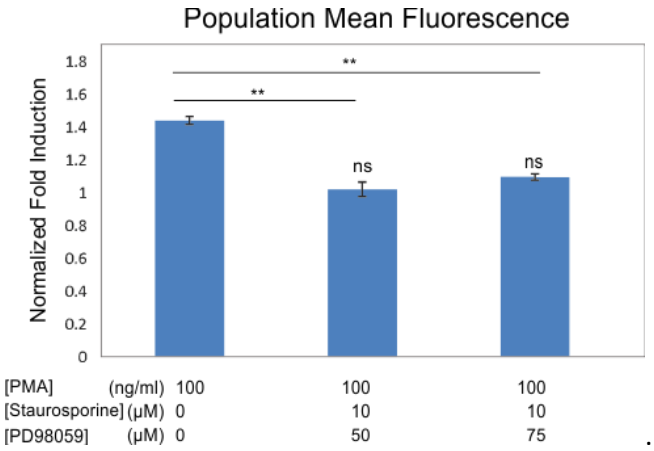


SI Figure 4. A. Induced fraction of activated cells 24h after PMA exposure. N = 3, error bars: standard error of mean. B. Representative histograms of control cells and PMA treated cells (100 ng/ml for 24h) shows population shift.

Inhibitor studies included blocking PMA based induction of PKC via staurosporine (SI Figure 5) and blocking PMA based induction of PKC and MEK using staurosporine and PD98059 together (SI Figure 6).

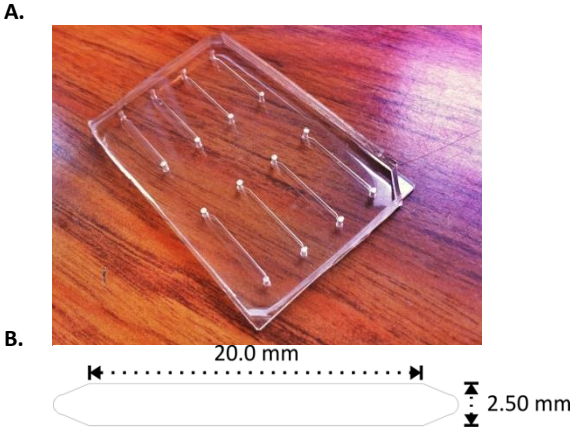


SI Figure 5. Inhibition of PMA induction using PKC inhibitor staurosporine. N = 3, error bars: standard error of mean.



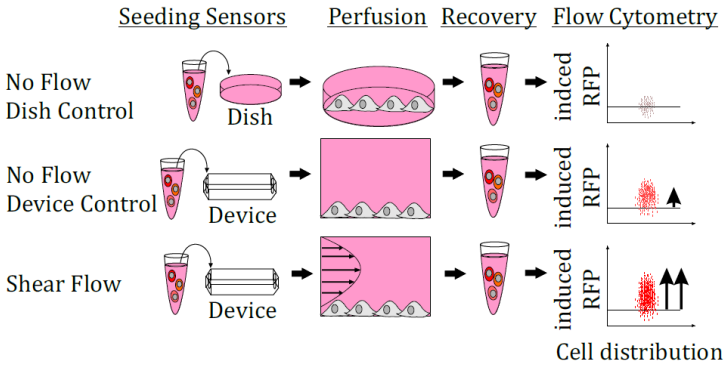
SI Figure 6. Inhibition of PMA induction using PKC inhibitor staurosporine and MEK inhibitor together. N = 3, error bars: standard error of mean.

Microfluidic perfusion device and individual channel geometry is depicted in SI Figure 7.

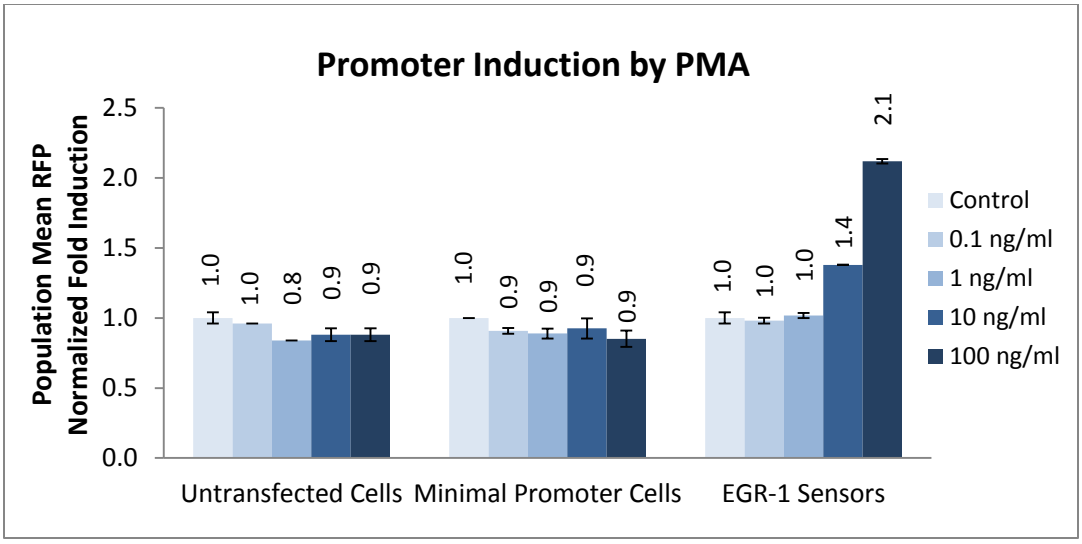


SI Figure 7. A. Image of microfluidic device for FSS studies. **B.** Channel geometry and dimensions.

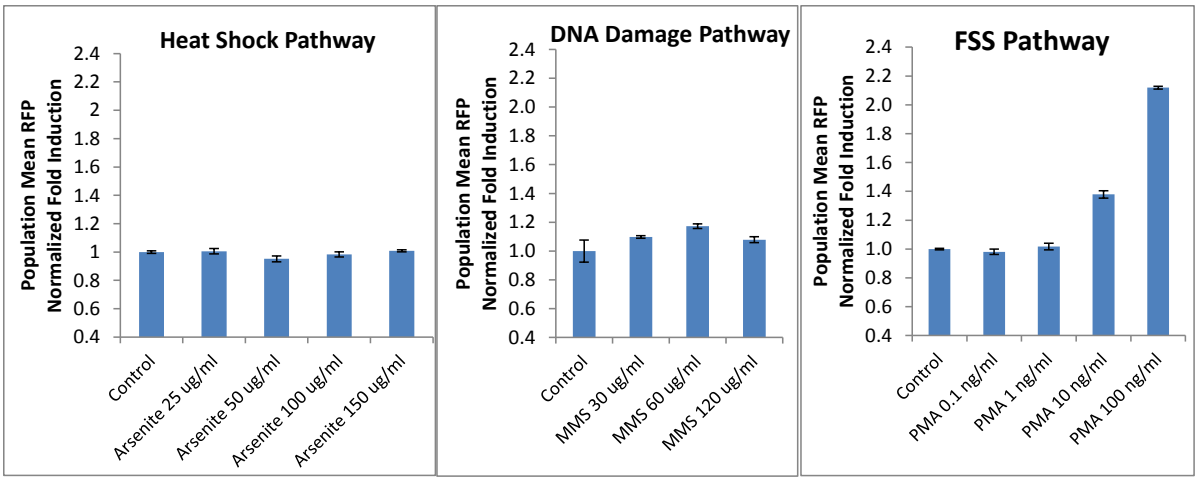
The workflow of FSS characterization of shear sensors in the microfluidic device is shown in SI Figure 8. Work flow consists of seeding cells in a device or dish overnight. Cells were perfused using syringe pump set at flow rates corresponding to desired FSS intensity. Cells were then recovered and seeded again for 24 hours before they were taken to flow cytometry analysis. Cells that experienced FSS were compared to no flow device control and their population mean RFP fluorescence was normalized to that control.



SI Figure 8. Workflow used FSS characterization of cells.



SI Figure 9. Promoter induction and validation. Non-transfected NIH3T3 cells and those transfected with a minimal promoter (non-inducible) plasmid were treated with 24h of varying PMA concentrations along with the EGR-1 sensor. Resulting RFP induction was normalized to untreated controls. N = 3, error bars: standard error of mean.



SI Figure 10. Cross reactivity of cell sensors. EGR-1 sensors were treated with varying concentrations of sodium arsenite for 30min, to activate heat shock; methyl-methanosulfate for 4hrs, to activate DNA damage; and PMA for 24hrs, to activate FSS pathway. Resulting RFP induction was normalized to untreated controls. N = 3, error bars: standard error of mean.

Guidelines for Sensor Analysis and Adaptation:

In regards to estimating an adequate cell number for estimating significant sensor signals, we performed statistical power analysis on our data. Specifically, given the means and variance of our control samples, we analytically computed the minimum fold induction that could be significantly resolved using a two sided t-test with an alpha value of 0.05, and with various power statistics. We have summarized our results below:

Minimally resolvable fold induction, given statistic power and a sample cell number:

		Cell number					
		10	**50**	**100**	**250**	**500**	**1000**
Power	**0.6**	1.805	1.327	1.229	1.144	1.102	1.072
	0.7	1.805	1.327	1.229	1.144	1.102	1.072
	0.8	1.909	1.369	1.258	1.162	1.115	1.081
	0.9	2.054	1.427	1.299	1.188	1.133	1.094

In order to interpret and use this table, one would first decide on a power of the statistic (probability to reject the null hypothesis: i.e. population means being equal, when the alternative hypothesis is true), when the significance level (probability of rejecting the null hypothesis when the null hypothesis is true) is 0.05. Next, according to the experimental context one would decide on a convenient cell number that could be assayed in their device. Using the table mentioned above, one would then find the minimum normalized mean-fold induction that could be resolved ($p<0.05$). For example, with a statistic power of 0.8, and alpha value of 0.05, with 10-50 cells one could resolve a ~2X fold induction, for instance relevant for analyzed PMA treated cells. We used our own PMA data to verify that with this range of cells we could indeed achieve significant differences, validating the table estimates. However, with these few cells, one would not be able to resolve FSS conditions as we tested in Fig. 5 or 6. Given the same statistical test parameters, for those conditions, one would conservatively require more than 500 cells.

While these estimates provide guidelines for picking minimum cell numbers for analysis, one still needs to still be careful in validating the significance of their data, and would be recommended to sample large cell numbers for improved readout sensitivity and significance. The technical variance in different assay methodologies may vary from that observed here. Additionally, biological variance needs to be taken into account; if all the cells come from one culture and were used in one device, that would not allow for substantial generalizability.