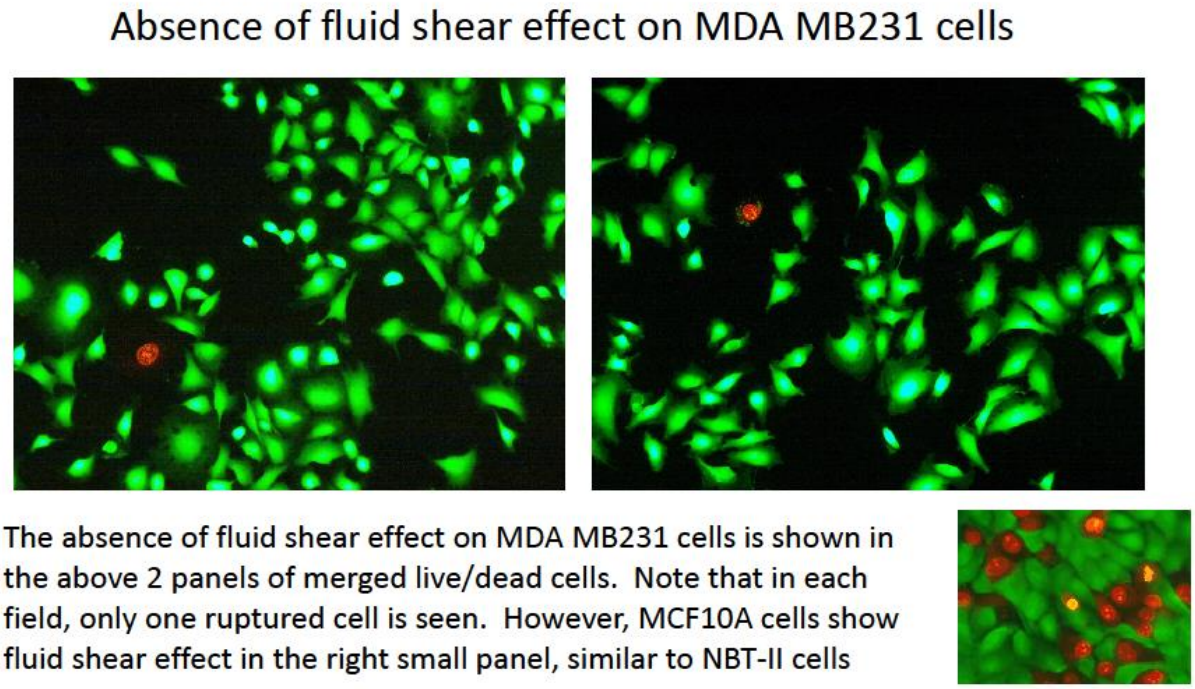
Additional File #1 (Tchao)

Fluid shear effect on MCF10A and MDA MB231 cells



These images present a cautionary note that comparing two different cell lines for expression of small molecules in the cytoplasm may yield uncertain results because if one cell line such as MCF 10A that responds to fluid shear with cell membrane rupture while another cell line such as MDA MB231 that does not respond to fluid shear.

Additional file # 2 (Tchao)

Methods and Materials:

NBT-II cells are routine culture in DMEM/F12 medium supplemented with 10% fetal bovine serum, all obtained from ThermoFisher.

HanksBalanced salt solution (HBSS) with Ca++ and Mg++ without phenol red, is obtained from ThermoFisher (cat number 14025092).

CalceinAM was obtained from Lifesciences as solids in 50µg/vial. Each vial is dissolved in 50µL DMSO to give a stock solution of 1mM concentration. For incubation with cells, the stock solution is diluted to 0.5-4 µM in HBSS. Preliminary experiments using 0.5µM CalceinAM have shown that DMSO from 0.1-0.4% do not affect CalceinAM uptake by cells.

CyQuant assay kit was purchased from Lifesciences , InVitrogen ® CyQUANT Cell Proliferation Assay Kit.

Anti GAPDH labelled with Alexa488 was purchased from ThermoScientific (cat #MA5-15738-D488) .

Zeiss Axioscope equipped with Polaroid color imaging system is used to record fluorescent images. NIH ImageJ program is used to merge fluorescent images.

Fluorescence is quantitatively measured in a plate reader, Cyto2300 at excitation 485nm and emission 590 nm.

Additional file #3 (Tchao)

Fluid shear Procedure applied to T-25 flasks:

Based on the preliminary results on CalceinAM loading, the following procedure has been adopted to measure the percentage of shear in T-25 flasks by various manufacturers.

Culture medium is aspirated from the T-25 flasks and 2ml cold HBSS is added to rinse the cells and then aspirated. Previous experiments have shown that cold HBSS does not produce the fluid shear effect of membrane rupture in cells [8, 9].

2ml CalceinAM, 2μM, is added to each T-25 flask and incubated at 37^{o}C for 60 minutes.

At the end of CalceinAM incubation, 2 ml Cold HBSS is added to each flask and extracellular CalceinAM is removed by aspiration, and the flasks are rinsed twice with 2 ml cold HBSS each time.

The second rinse of 2ml HBSS is kept for fluorescence measurement, that serves as the background for the specific flask to calculate for the non-shear % of the flask.

Then, warm HBSS 2ml is added and the flask is incubated at 37^{o}C for 10 minutes to equilibrate the warm HBSS solution and the flask. Fluid shear is applied to the cells by rocking the flask back and forth, and the fluid is collected for fluorescence measurement, representing the fluorescence of sheared cells. After the removal of the "fluid shear" solution, 2 ml of 0.1% Triton X100 in HBSS neutralized to pH 7.2, is added and incubated at room temp for 10 minutes to release the total fluorescent Calcein in the remaining cells. The Triton solution is collected for fluorescence measurement. All fluorescence measurements were done in a fresh 24 well plate.

The following formula is used to calculate the percent of shear in each flask :

Sheared cell washings + Triton X100 released fluorescence = Total fluorescence

Fluid Sheared cell solution fluorescence / Total fluorescence = % heterogeneity on PS surface

For each run, 3-4 flasks of each brand are used. The experiments are repeated 3x (n=3) for statistical analysis of significance.

Additional file #4 (Tchao)

Data to characterize the kinetics of CalceinAM uptake by NBT-II cells

Figure 3 CalceinAM uptake by NBT-II cells

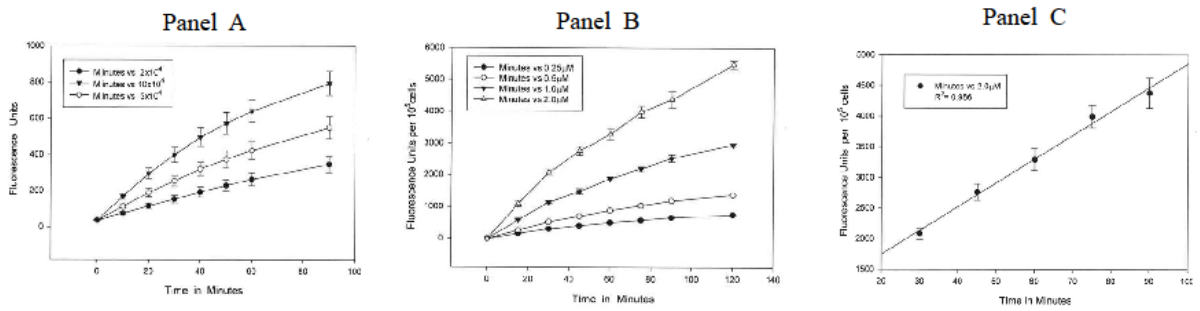


Figure 3 shows the kinetics of CalceinAM loading in NBT-II cells.
Cells are seeded in 24-well plates, after the addition of CalceinAM, the fluorescence in each well is measure at 485nm excitation and 590nm emission wavelengths.
Panel A shows that the uptake over time is cell density dependent.
Panel B shows that the fluorescence in cells is dependent on CalceinAm concentrations
Panel C shows that using 2μM CalceinAM, the uptake at various cell densities is linear over 90 minutes. Therefore, for comparing various flasks, 2μM CalceinAM is used.

Triton extraction data :

In order to measure the total Calcein fluorescence in each flask, the cells were extracted with 0.1% neutral TritonX100. To test the efficiency of extracting total Calcein fluorescence in cells, cells are grown in a 24-well plate to 70-80% confluent cultures. Cells are then loaded with CalceinAM (2-5 μM) in 1ml HBSS. The Calcein fluorescence is measured in a plate reader. After the removal of the CalceinAm incubation solution, the wells are rinsed with cold HBSS. The total Calcein fluorescence in the cells is extracted completely with Triton X100 as represented by percent of Calcein fluorescence measured in the 24-well plate and the results are shown in Table 2.

Table 2: Triton extraction of preloaded CalceinAM fluorescence.

	Triton extraction %	SD
Expt 1	98.8	0.04
Expt 2	102.7	0.09
Expt 3	95.0	0.13

Table 2 legend: Triton extracted fluorescence was compared to the fluorescence in monolayer cells in 24 well plates, after 60 minutes incubation with 2μM CalceinAM. Each experiment consists of 12 wells of cells. After rinsing the cells with cold HBSS, 1ml 0.1% neutralized Triton x100 was added. After incubation at room temp for 10 minutes, the triton solutions were removed into fresh wells for fluorescence measurements.