Electronic Supplementary Material (ESI) for Nanoscale Advances.
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Supporting Information

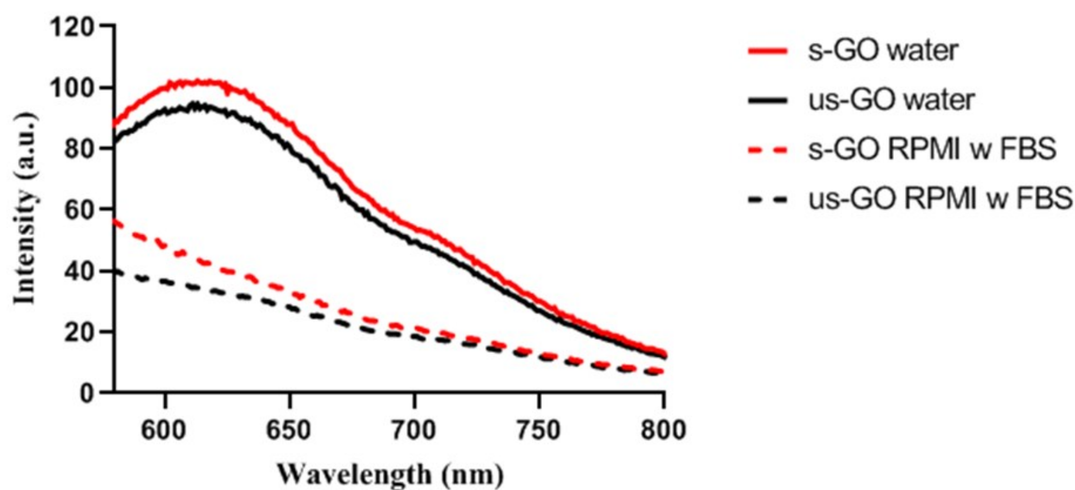
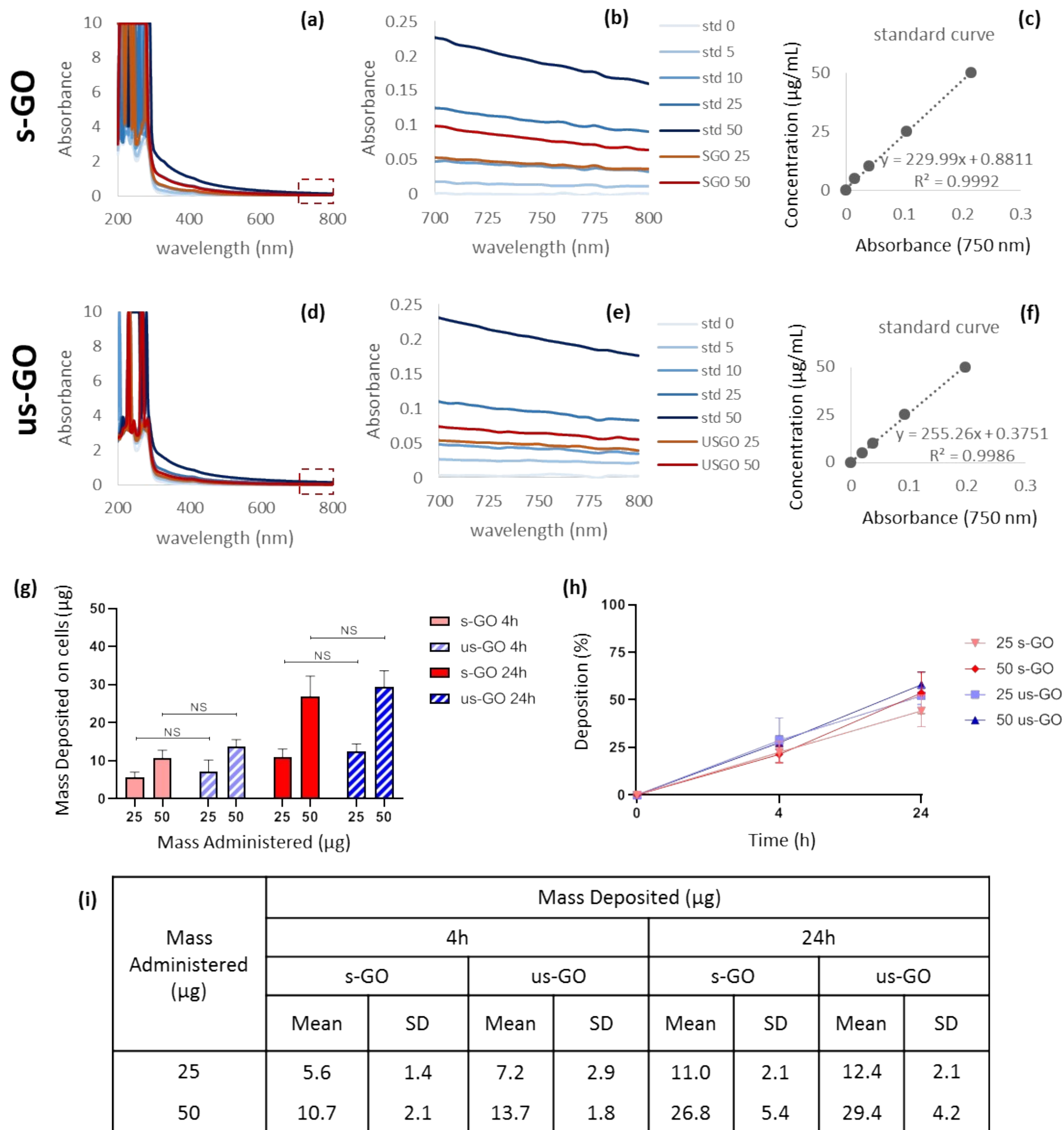


Figure S1. Spectrofluorometric analysis of the emission spectra of s-GO and us-GO (2 mg/mL) dispersed in water (solid lines) and in RPMI with 10 % serum (patterned lines – samples prepared in RPMI with serum were re-suspended in water for the measurement) using an excitation wavelength of 525 nm. This result shows that s-GO retained a higher intensity of intrinsic fluorescence comparing to us-GO in both water and cell culture medium with serum. The lower fluorescence intensity of the material in the cell culture medium can be explained by the agglomeration of material in the culture medium.



Mass Administered (µg)	Mass Deposited (µg)							
	4h				24h			
	s-GO		us-GO		s-GO		us-GO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
25	5.6	1.4	7.2	2.9	11.0	2.1	12.4	2.1
50	10.7	2.1	13.7	1.8	26.8	5.4	29.4	4.2

Figure S2. GO deposition measurement on BEAS-2B cells**.** To evaluate the mass of s-GO and us-GO deposited on BEAS-2B cells, supernatants were collected at 4 and 24 hours and the absorbance was measured using UV-VIS absorption spectroscopy. UV-vis spectra of (**a** and **b**) s-GO and (**d** and **e**) us-GO at concentrations ranging from 0 to 50 µg/mL is showed, with enlarged spectra at region of interest (**b** and **e**). The concentrations of materials remaining in suspension were determined thanks to standard curves (**c** and **f**), based on the absorbance values measured at 750 nm. The mass of material deposited on cells (**g**) were then calculated based on the difference between the mass of material administrated and remaining in suspension (*Mass Deposited* = *Mass Administrated* – *Mass remained in suspension*). Percentages of deposition (**h**) were also calculated. Difference of deposition between s-GO and us-GO were evaluated using one-way ANOVA followed by Dunnett's post hoc test

(minimum of 3 independent experiments). No significant differences in mass deposited were observed between the s-GO and us-GO. (*std = standard, SD = standard deviation, NS = not statistically significant*)

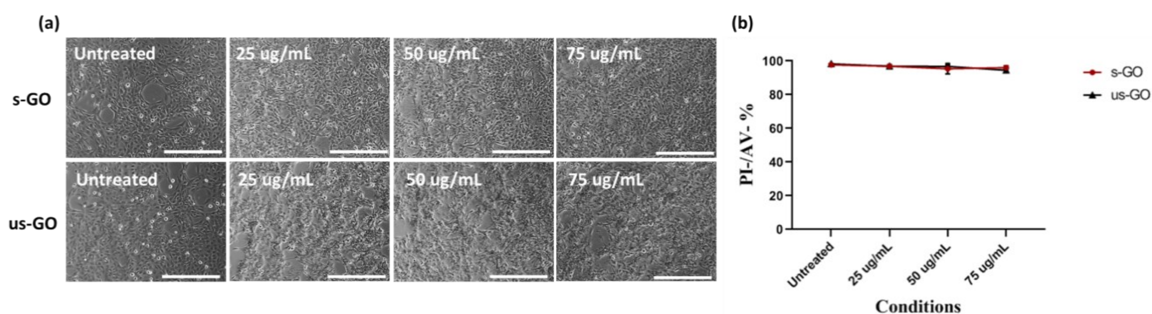
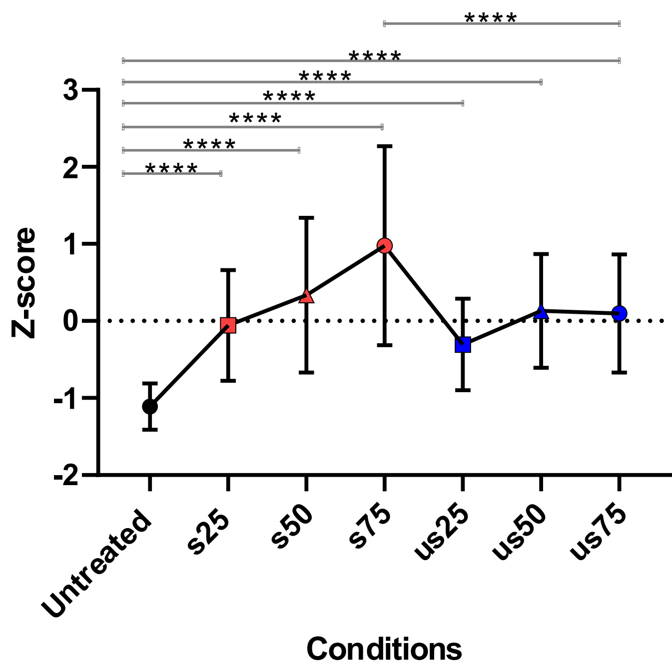


Figure S3. Cytotoxicity of s- and us-GO in BEAS-2B (24 h treatment) was assessed *via* (**a**) optical imaging, and (**b**) propidium iodide (PI)/ annexin V (AV) staining using flow cytometry (positive control is shown in **Figure S10**). Representative optical images of BEAS-2B cells treated with and without s- or us-GO are showed. And we observed no obvious changes in cell morphology and cell confluence with the treatment of GO compared to the untreated cells. The PI/AV assay confirmed no significant difference in the percentage of live cells (i.e. cells unstained by both PI/AV (PI-/AV-)) with the treatment of GO compared to the untreated cells. Scale bar = 400 μm.



Conditions	Number of cells analysed
Untreated	47
s25	39
s50	41
s75	43
us25	45
us50	46
us75	48

Figure S4. Standardised quantitative analysis of the fluorescent images for the interaction of GO with BEAS-2B cells. The results showed the uptake of GO in treated samples (regardless of size and concentrations) are all significantly greater than untreated samples. And the result showed no statistical differences in the uptake of s- and us-GO except for the highest concentration of 75 μg/mL. The images were manually analysed using ImageJ. The data were statistically analysed using analysis of variance (one-way ANOVA) and Tukey's multiple comparison test with **** equate to $p < 0.0001$. n = 3 independent experiments. s = s-GO, us = us-GO, 25 = 25 μg/mL, 50 = 50 μg/mL and 75 = 75 μg/mL.

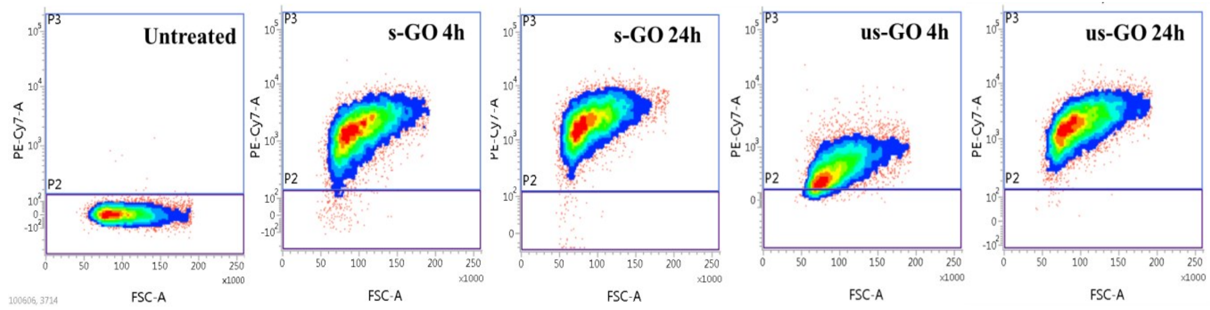


Figure S5. FACS density plots of untreated and BEAS-2B cells treated with 75 μg/mL of s-GO or us-GO at 4 h and 24 h time points. PE-Cy7-A channel was used to detect the auto-fluorescent signal from the GO. Cellular interaction with GO was assessed by flow cytometry by measuring the auto-fluorescent signal of GO in the PE-Cy7-A channel. It is obvious that the intensity of fluorescence of us-GO is much lower at 4 h compared to 24 h, whereas for s-GO the intensity of fluorescence is only slightly lower at 4 h compared to 24 h.

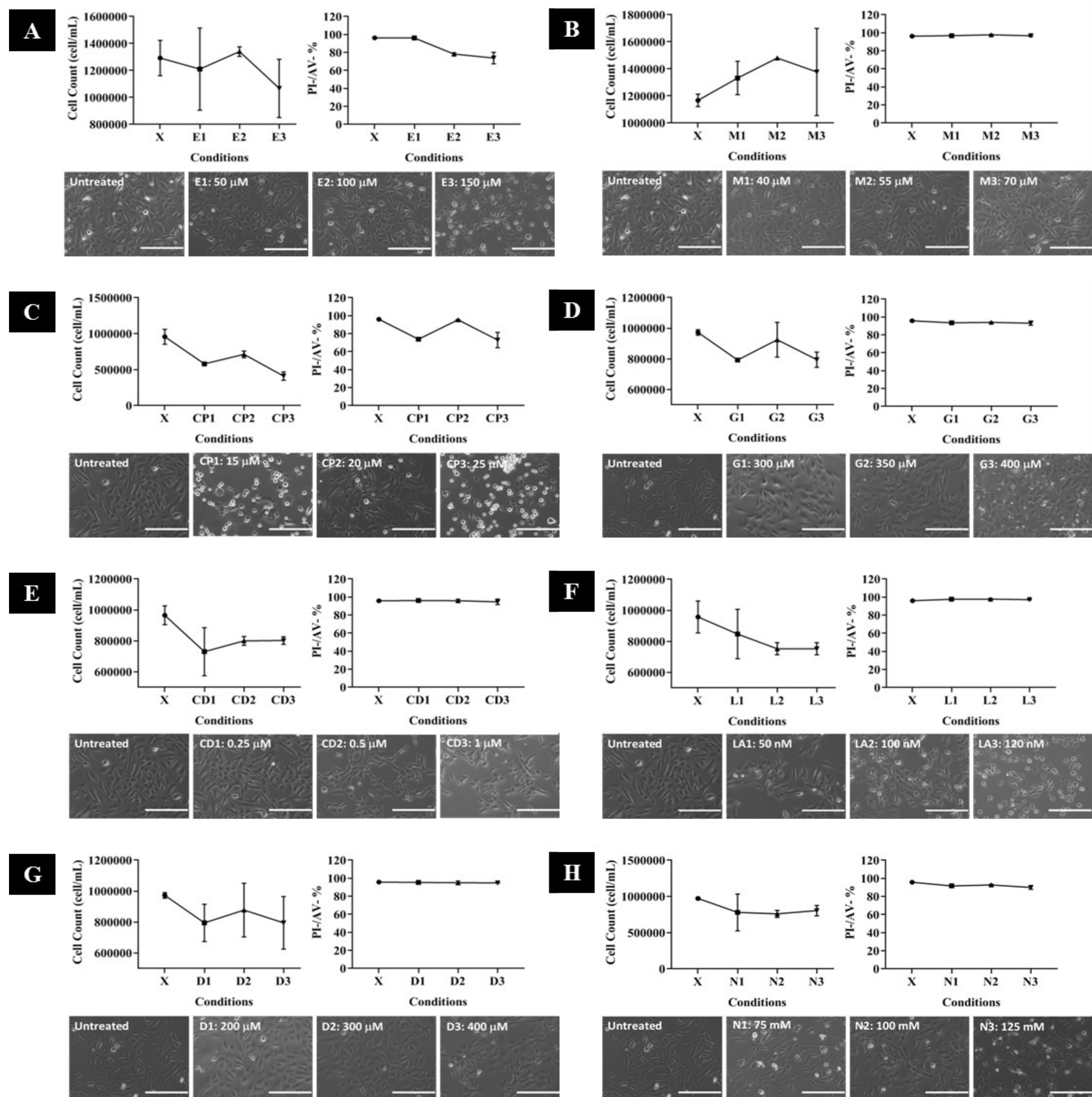


Figure S6. Cytotoxicity of each inhibitor using three different concentrations was assessed *via* optical imaging, cell counting by Trypan Blue dye exclusion assay, and propidium iodide (PI)/ annexin V (AV) staining using flow cytometry (positive control is shown in **Figure S10**). The optical images enabled to assess changes in cell morphology and healthiness of the monolayers, while live cell counting and PI/AV bivariate plots quantified cytotoxicity induced by the treatment with inhibitors. PI will stain for early necrotic cells, whereas AV will stain for early aprotic cells, cells which stained by both PI and AV indicate late cell death and cells unstained by both PI/AV are live cells (PI-/AV-). The selected working concentration for each inhibitor is further tested for disruption to actin filaments (**Figure S7**). Scale bar = 200 μm. (**A**: Ethyl-isopropyl amiloride, **B**: Monodansylcadaverine, **C**: Chlorpromazine, **D**: Genistein, **E**: Cytochalasin D, **F**: Latrunculin A, **G**: Dynasore and **H**: Sodium azide)

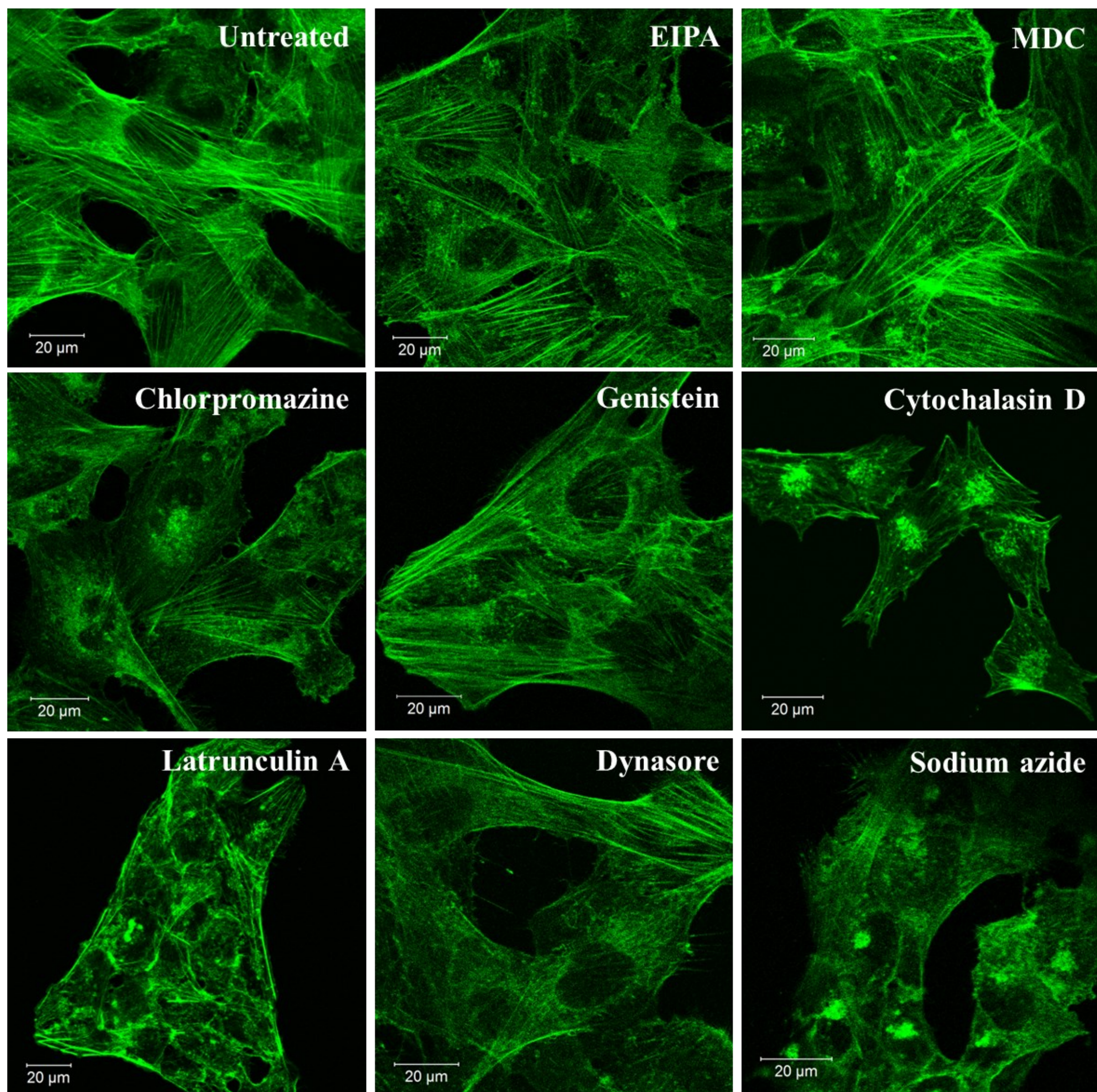
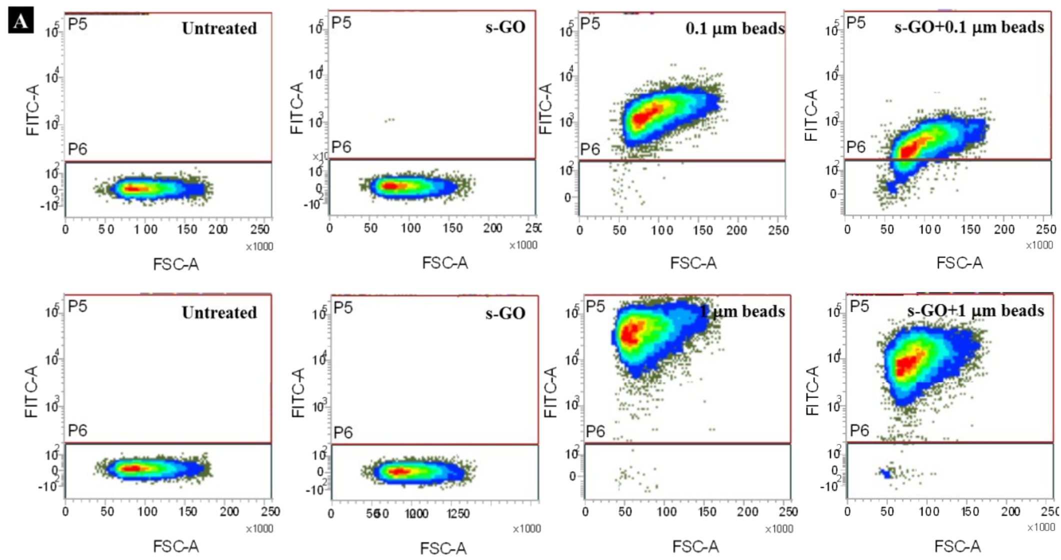


Figure S7. Actin filament staining of BEAS-2B cells with inhibitor at the selected working concentration (**Table 2**) using confocal microscopy. The result shows that in general the inhibitors caused no disruption of actin filaments, except for Cytochalasin D and Latrunculin A. Green = actin filaments.



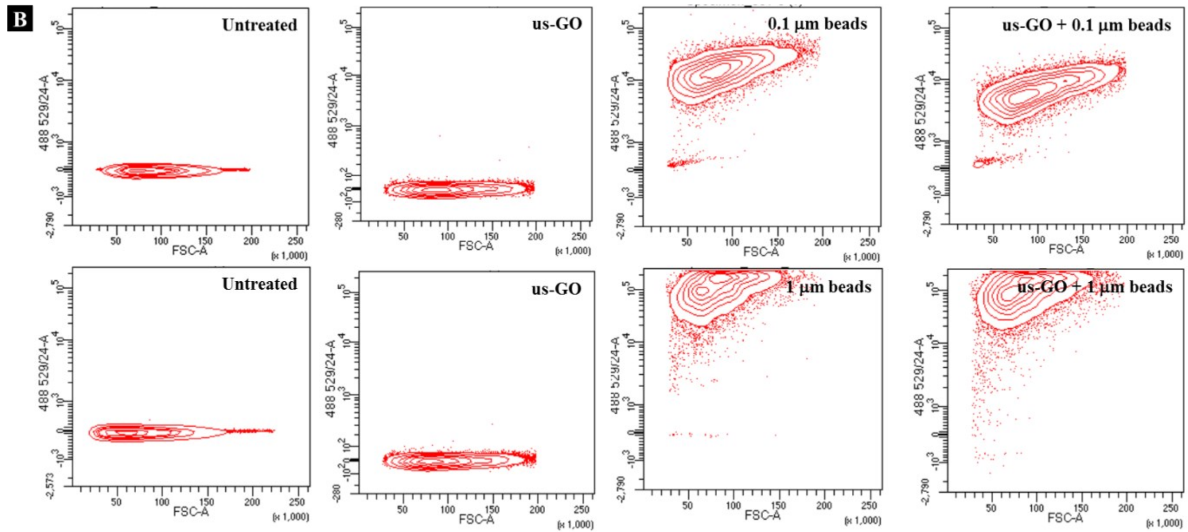


Figure S8. FACS density plot of untreated BEAS-2B cells, BEAS-2B cells treated with only GO, beads (0.1 or 1 μm beads), or beads (0.1 or 1 μm beads) in the presence of (**A**) s-GO, acquired using FACSVerse or (**B**) us-GO, acquired using Fortessa X20 at 50 μg/mL. This figure confirms that the fluorescent signal of the 0.1 and 1 μm beads changes in the presence of s-GO and us-GO, but to a different extent. Fluorescent signal of GO only was comparable to untreated cells.

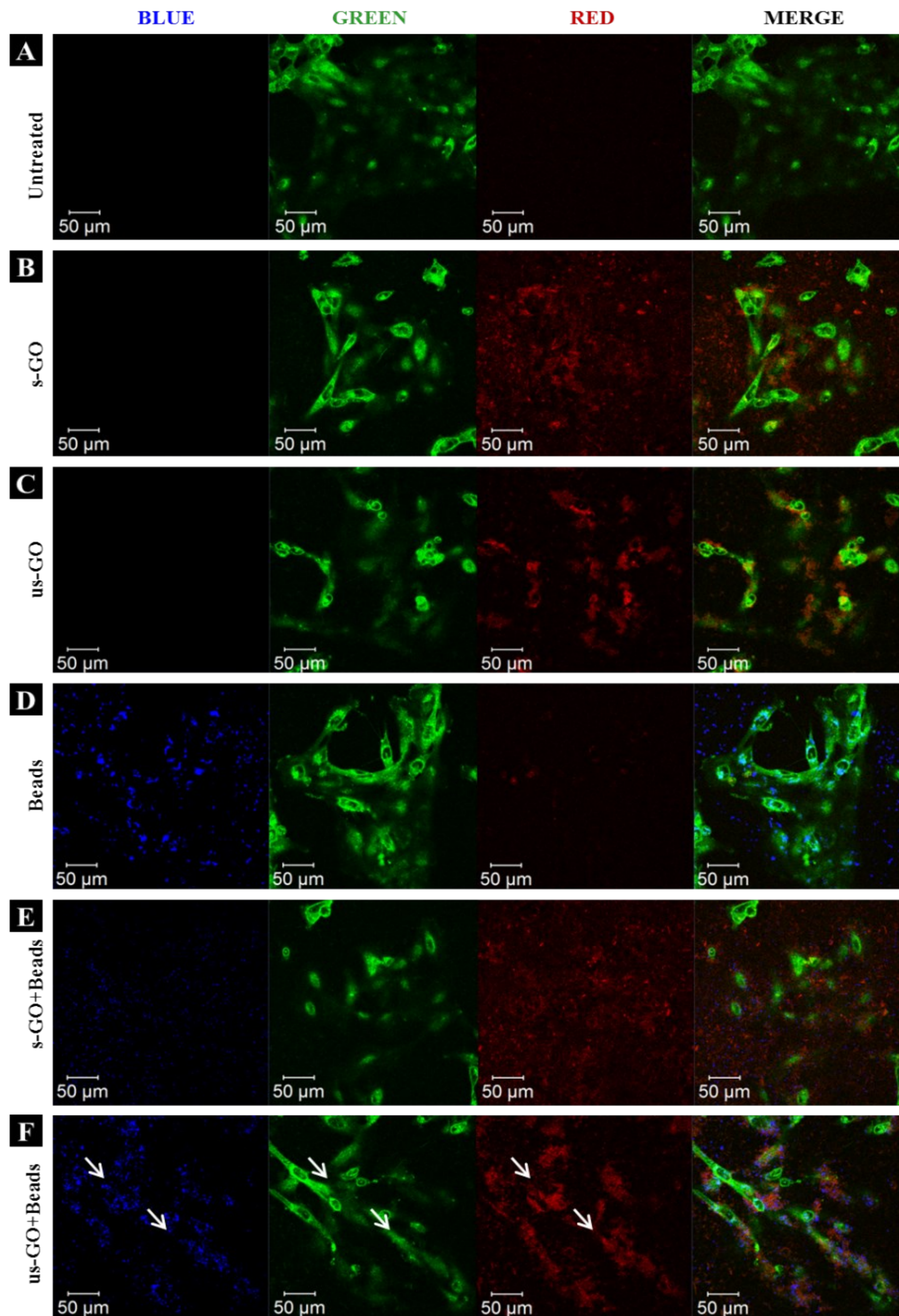


Figure S9. The apical section of BEAS-2B cells treated with 1 μm beads in the presence/absence of s-GO/us-GO (50 μg/mL). White arrows indicate regions co-localisation between the beads and us-GO on top of the cells (**F**), which was not observed for cells treated with s-GO + beads (**E**). (**A**: untreated cells, **B**: s-GO treated, **C**: us-GO treated, **D**: beads treatment, **E**: s-GO + beads, **F**: us-GO + beads) Green = plasma membrane, red = GO, blue = beads.

Table S1. Summary of the sizes and Zeta potentials of the materials (in RPMI w FBS) used in the study. Materials were prepared in RPMI w FBS and measured within 1 hour of sample preparations. The result indicated the 0.1 and 1 μm beads had a similar surface charge to the GO we produced.

Material	Average hydrodynamic diameter (d.nm)	Polydispersity index	Zeta potential (mV)
FITC-labelled carboxylate-modified 0.1 μm bead	135.7 ± 0.902	0.233 ± 0.002	-10.1
FITC-labelled carboxylate-modified 1 μm bead	841.1 ± 47.77	0.507 ± 0.102	-10.2
Blue fluorescent carboxylate-modified 1 μm bead	615.6 ± 46.91	0.492 ± 0.064	-9.89
s-GO	652.9 ± 101.6	0.823 ± 0.158	-11
us-GO	170.3 ± 0.8083	0.484± 0.002	-10.8

FITC-labelled = fluorescein isothiocyanale-labelled

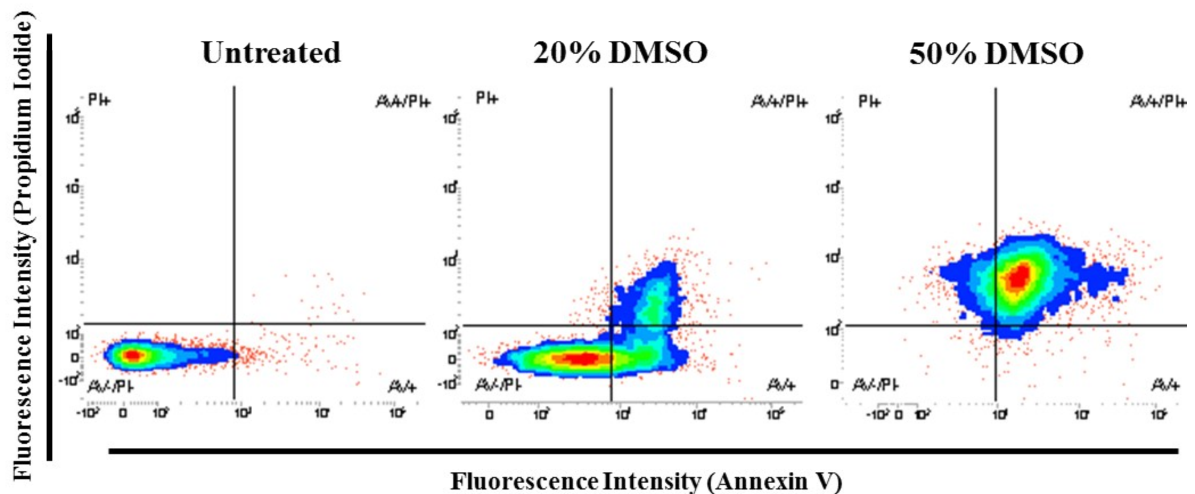


Figure S10. Dimethyl sulfoxide (DMSO) is used as the positive control in propidium iodide (PI)/ annexin V (AV) staining of BEAS-2B cells.