

## Supplementary Materials

### **Substrate stiffness modulates extracellular vesicles' release in a triple-negative breast cancer model**

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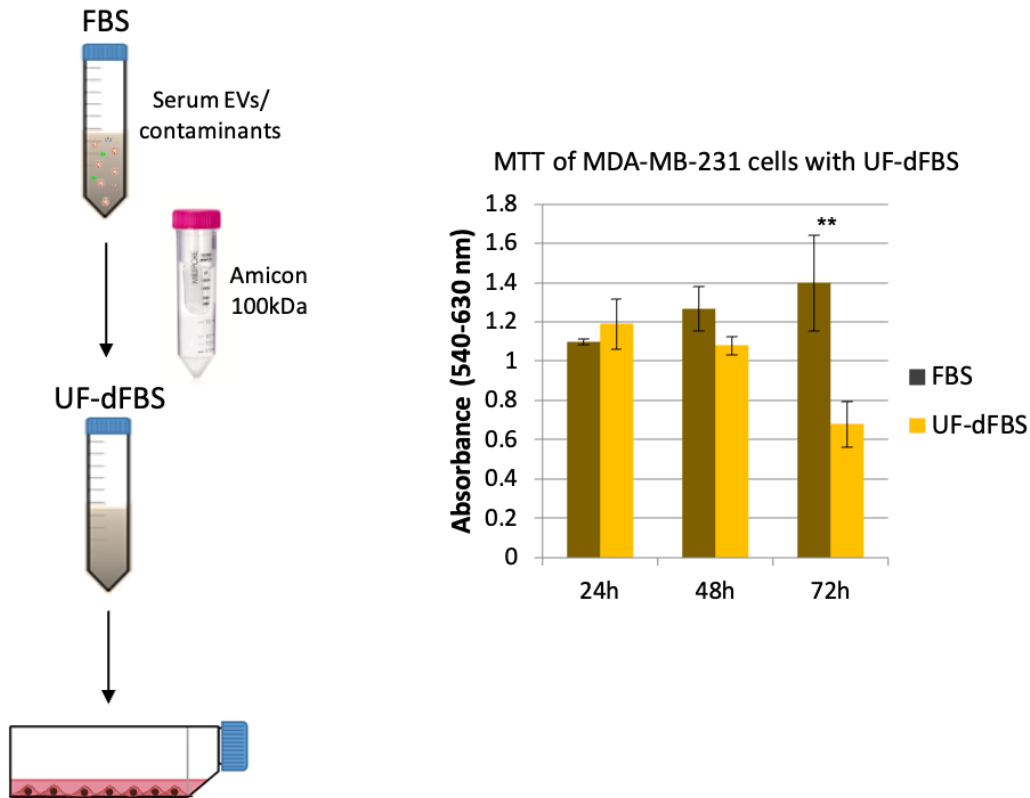
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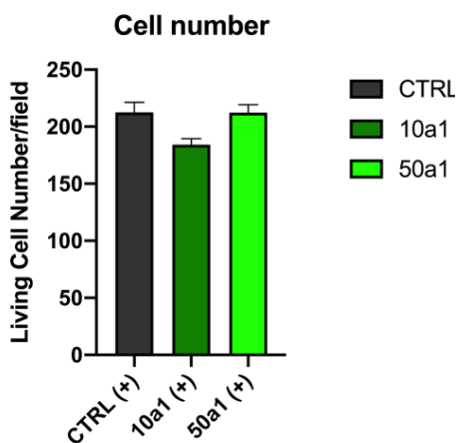
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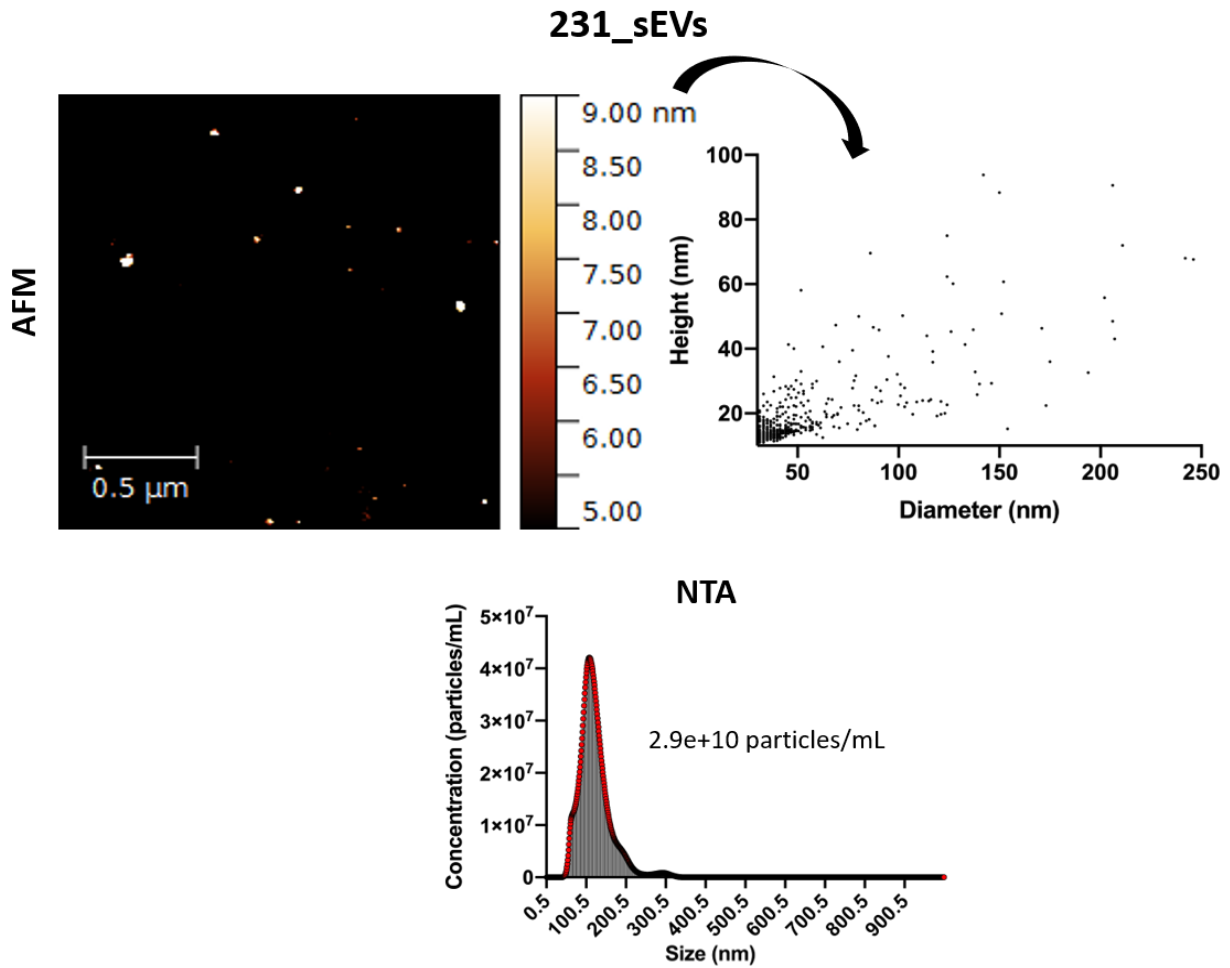
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**Supplementary Figure 1.** MTT assay showing the cell vitality of MDA-MB-231 cells grown with UF-dFBS for 1,2,3 days. Significance of data differences was established via two-tailed Student's t-test. UF-dFBS: Ultrafiltrated EV-depleted FBS.

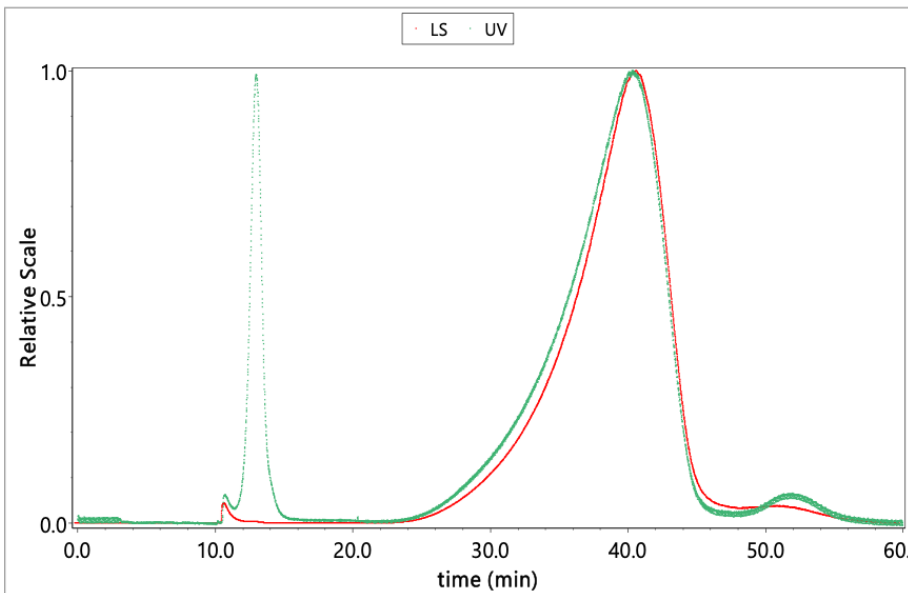
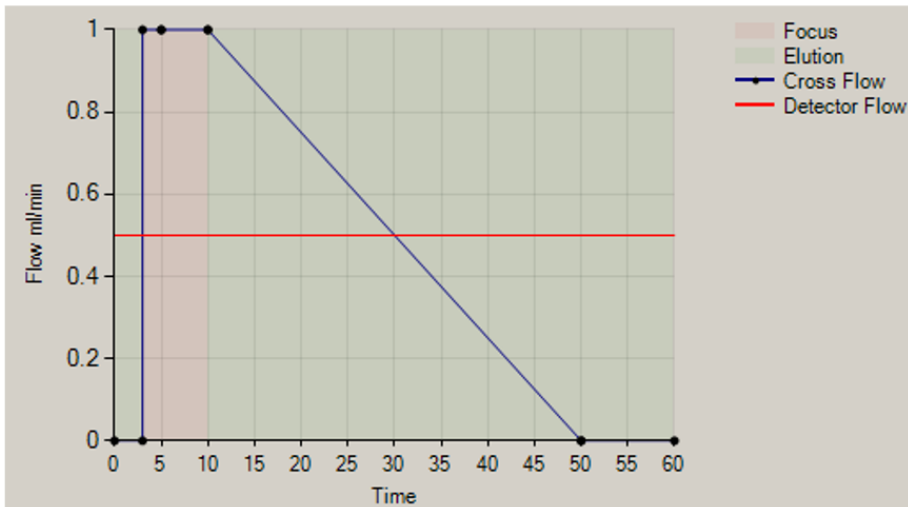


**Supplementary Figure 2.** Cell Number assay showing the cell proliferation of MDA-MB-231 cells grown on substrates at different stiffness. Significance of data differences was established via Ordinary one-way Anova test.

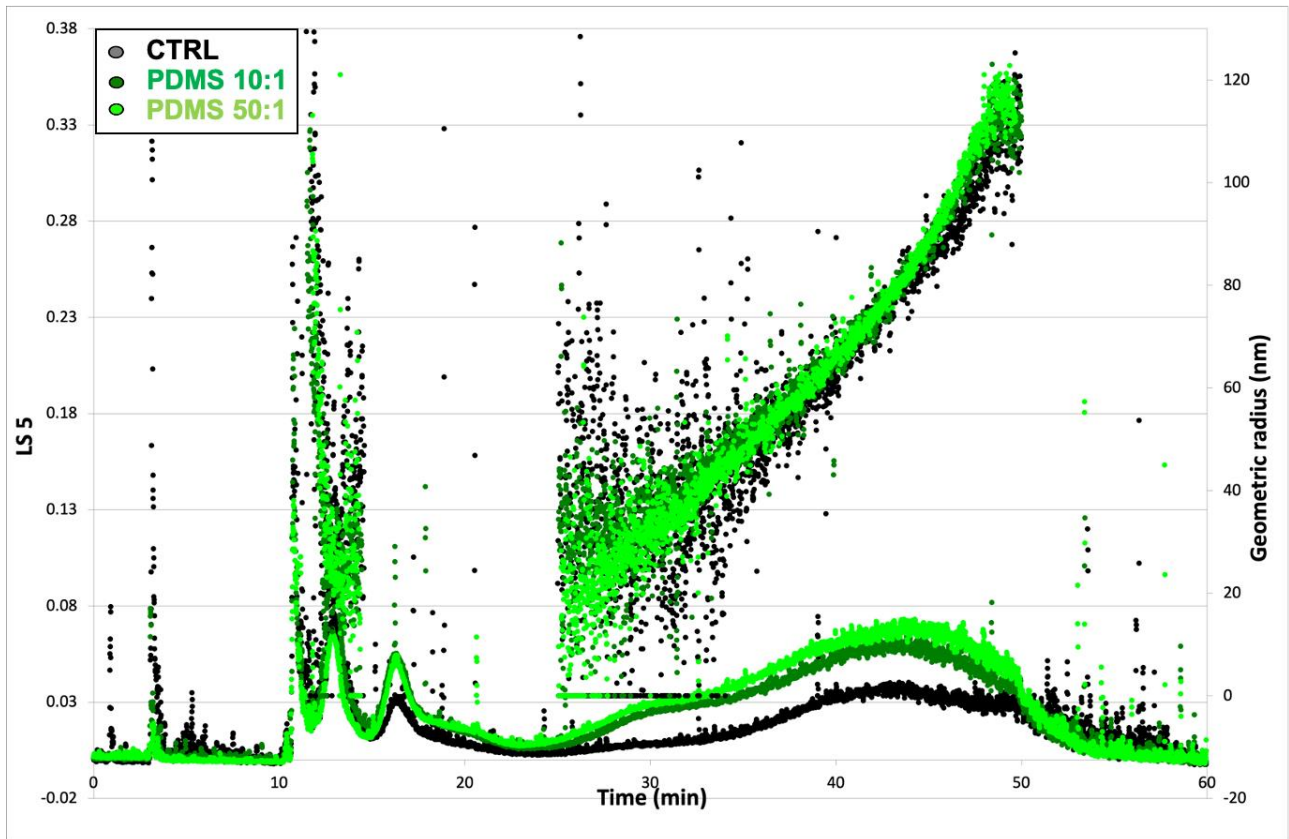


**Supplementary Figure 3.** MDA-MB-231-derived sEV (231\_sEVs) characterization: a representative atomic force microscopy image, scatterplot showing height and diameter obtained from the analysis of AFM images, and NTA. AFM: Atomic force microscopy; NTA: nanoparticle tracking analysis.

## Method flow diagram



**Supplementary Figure 4.** Method flow diagram used for the AF4-MALS measurements and the AF4-MALS fractogram of the reference material (BSA + liposomes) using this method (on bottom). The graph shows the MALS signal ( $90^\circ$  scattering angle, LS 5) in red and the UV absorbance at 280 nm in green. AF4: Asymmetric flow field-flow fractionation; MALS: multi-angle light scattering.



**Supplementary Figure 5.** AF4-MALS fractograms of 231\_sEVs stored at  $-80^{\circ}\text{C}$ . AF4: Asymmetric flow field-flow fractionation; MALS: multi-angle light scattering.