

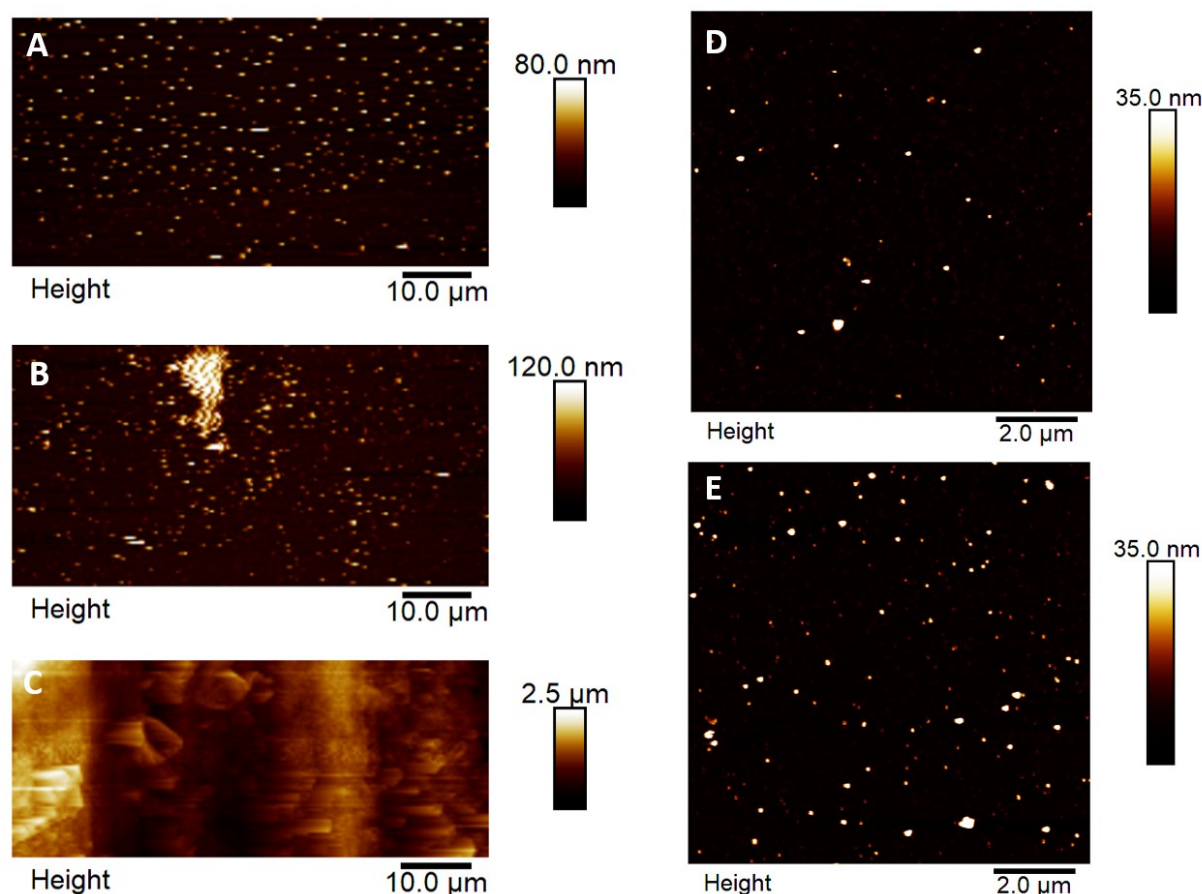
## SUPPORTING INFORMATION

### High content, quantitative AFM analysis informs scalable biomechanical properties of Extracellular Vesicles.

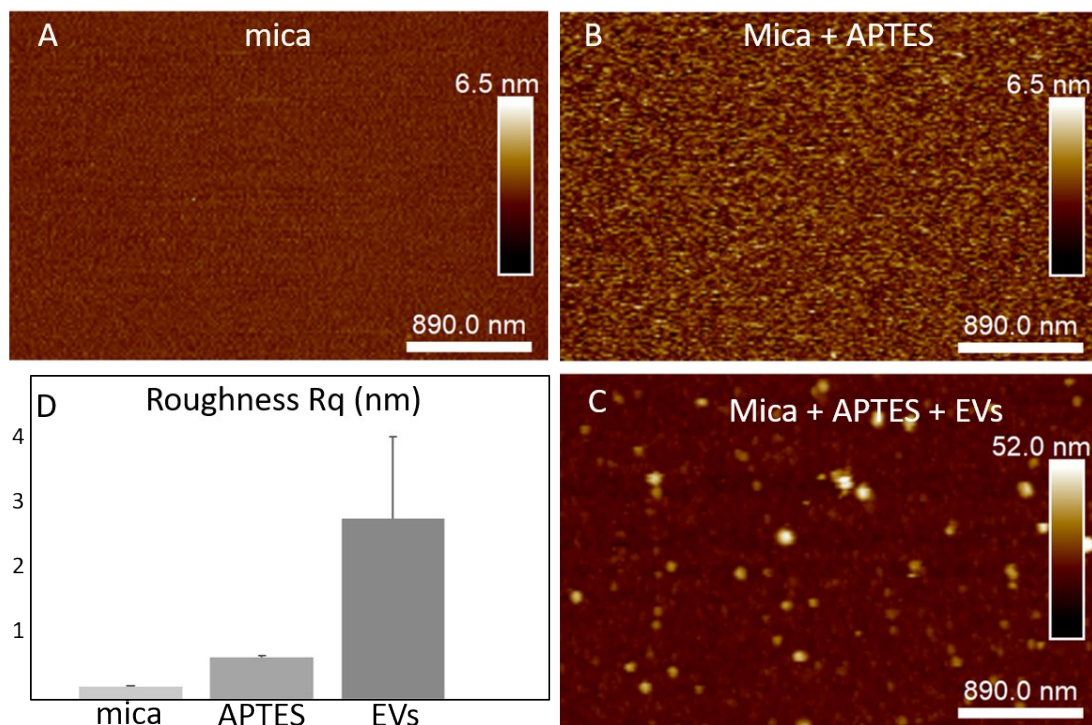
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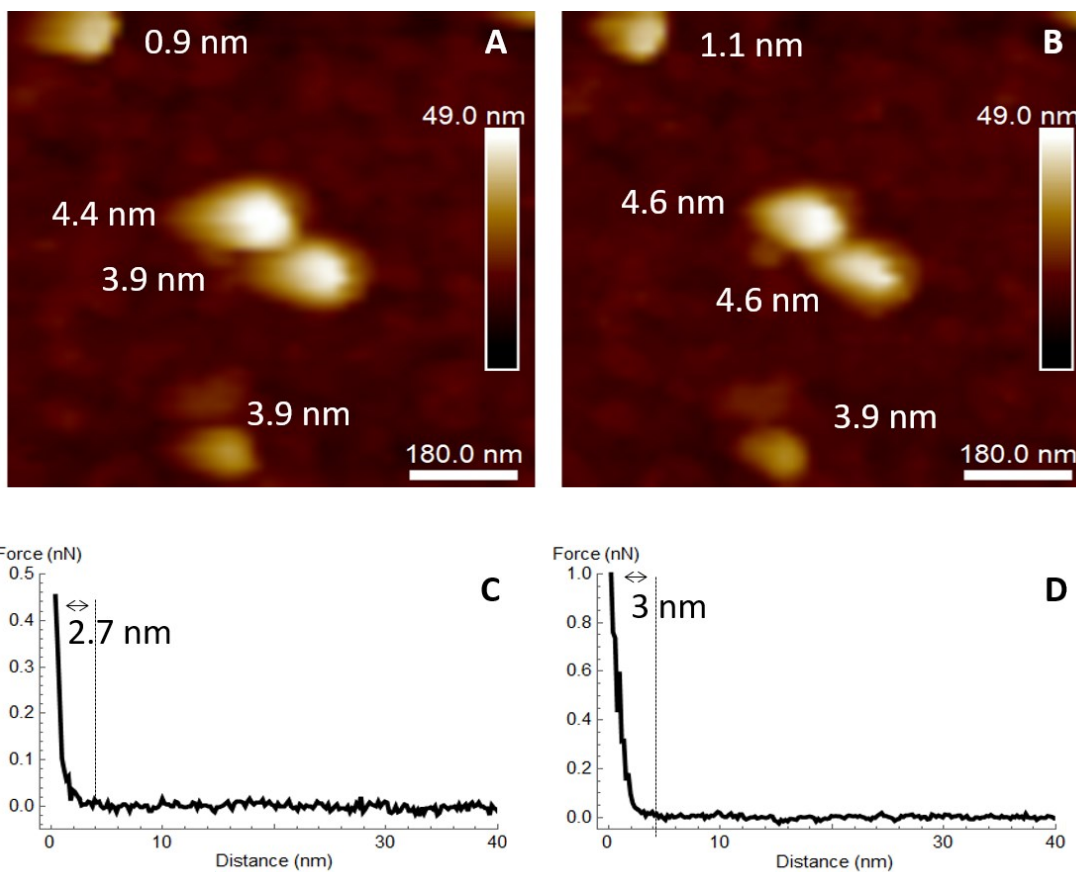
### Supporting Figures



**Figure S1. EVs Immobilization.** (A-C) Deposition of EVs on different substrates: mica (A), glass (B), PTFE (C). The higher roughness in PTFE does not allow a clear identification of EVs. (D-E) Comparison of EVs density on freshly cleaved mica (D) and on mica treated with APTES (E).

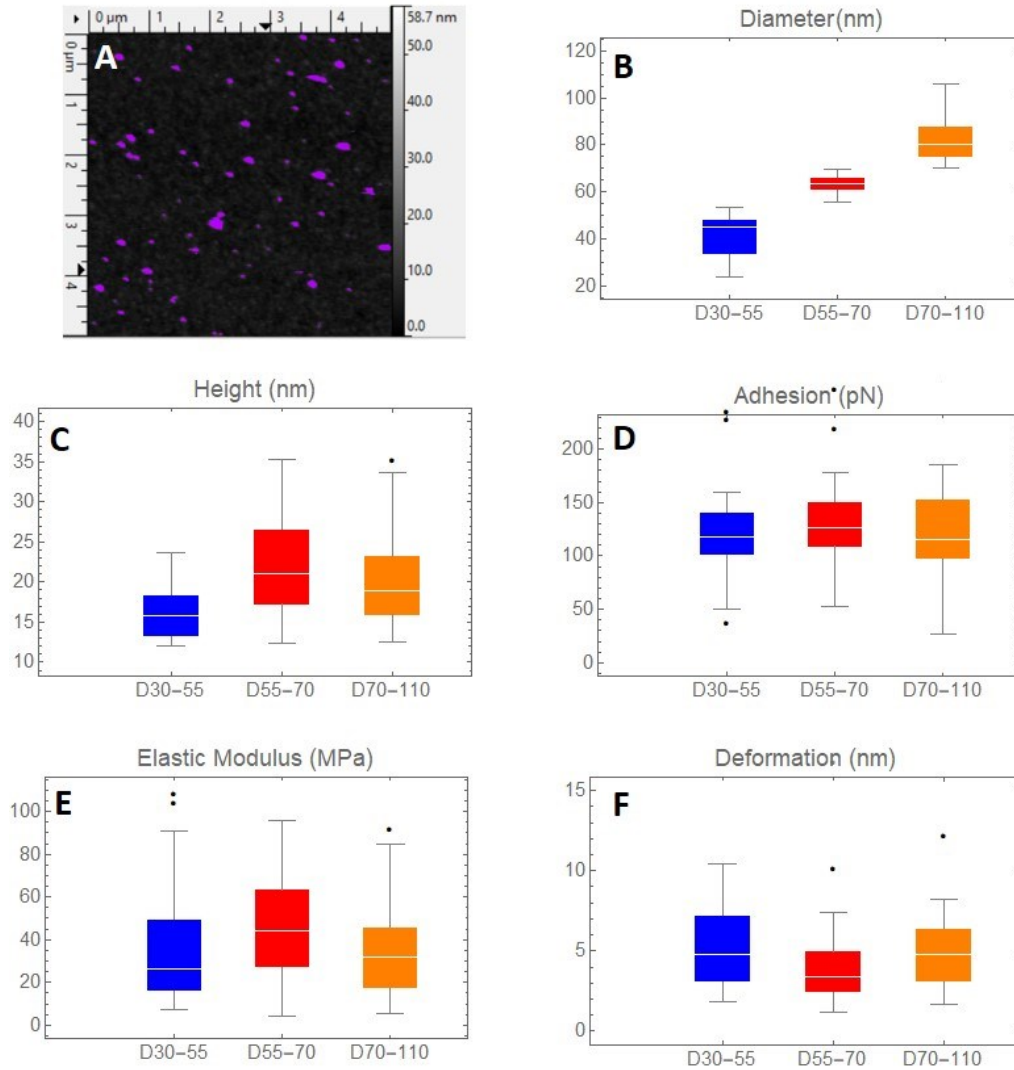


**Figure S2. Effect of APTES and EVs on mica topography.** In A is shown mica following cleavage, while in B mica after APTES deposition. C displays a mica area functionalized with APTES and following EVs deposition. D) Roughness Rq values of bare mica ( $R_q = 0.19 \pm 0.01$  nm), of APTES on mica ( $R_q = 0.64 \pm 0.02$  nm) and EVs on mica functionalized with APTES ( $R_q = 2.8 \pm 1.2$  nm). All values significantly different ( $p < 0.05$ ).



**Figure S3. Effect of scanning settings on EVs.** Data on the left (A) show a scanned area with 500 pN force, while the one on the right is obtained with 1 nN (force). The use of 500 pN is not sufficient to properly scan EVs, while using a 1 nN results in an evident better scanning of the sample. The use of higher force results in a low increase in particles deformation. (C) and (D) displays representative force curves and EV indentation at 0.45 and 1 nN, respectively.

## Control EVs



**Figure S4. Effect of size on EVs properties in control EVs.** (A) Nanomechanical properties when grouped in three diameter ranges for control EVs. A  $25 \mu\text{m}^2$  AFM area is shown in A, where EVs are identified with Gwyddion thresholding algorithm (EVs positions masked in purple). Diameter is shown in panel (B), height in (C), adhesion in (D), elastic modulus in (E) and deformation in (F). Diameter range is indicated in the x-axis: D30-55= diameter between 30 and 55 nm; D55-70= diameter between 55 and 70; D70-110=diameter between 70 and 110 nm.