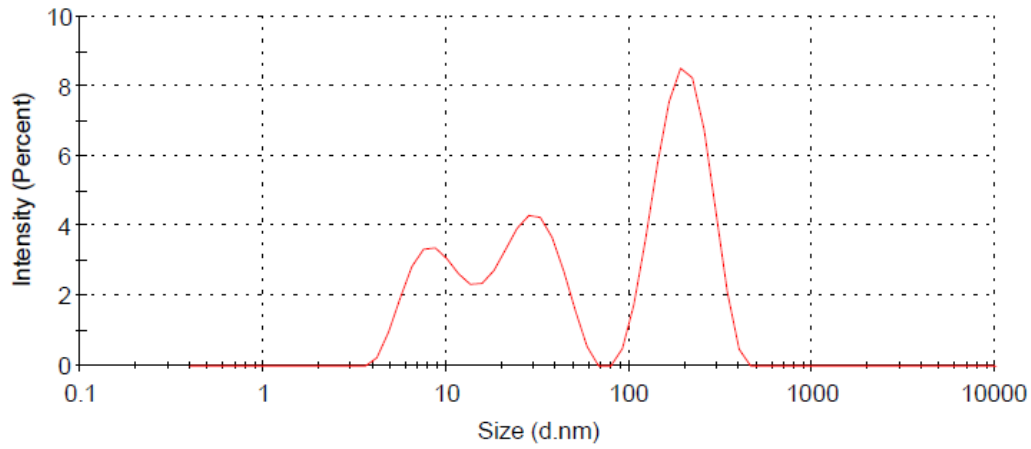


Supplementary Information.

a

Size Distribution by Intensity

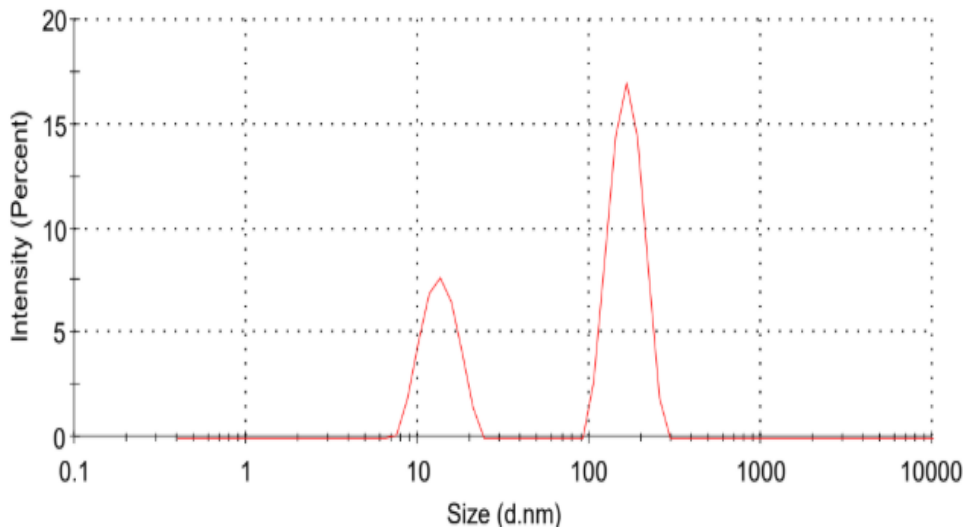


Results

	Size (d.nm)	% Intensity	St Dev (d.nm)
Z-Average (d.nm): 98.96	Peak 1: 203.4	48.6	63.22
PdI: 0.265	Peak 2: 28.81	31.0	10.90
Intercept: 0.914	Peak 3: 8.819	20.3	2.603

b

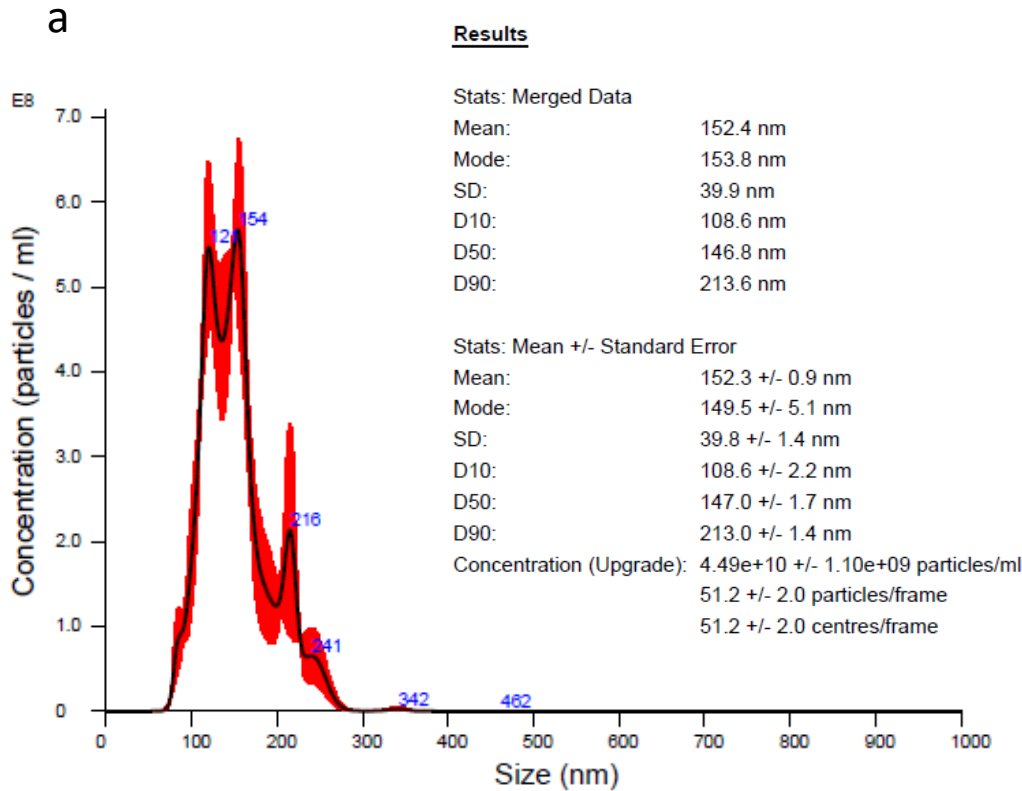
Size Distribution by Intensity

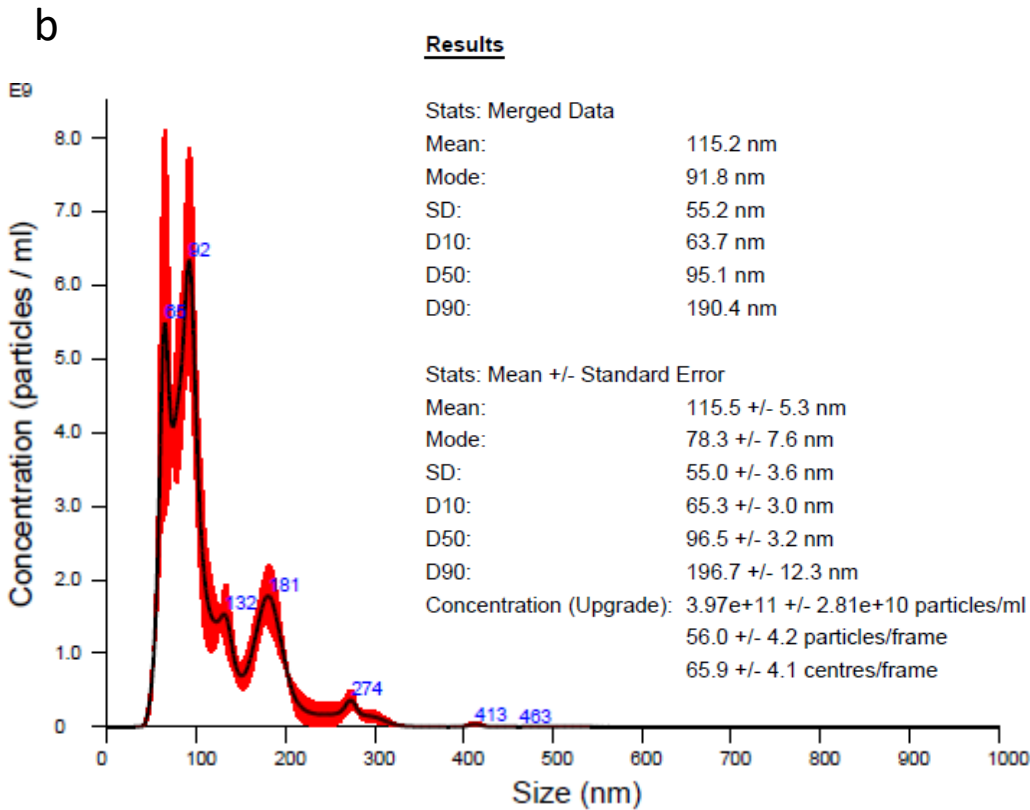


Results

	Size (d.nm)	% Intensity	St Dev (d.nm)
Z-Average (d.nm): 507.0	Peak 1: 166.6	66.8	34.88
PdI: 0.540	Peak 2: 13.69	33.2	3.125
Intercept: 0.404	Peak 3: 0.000	0.0	0.000

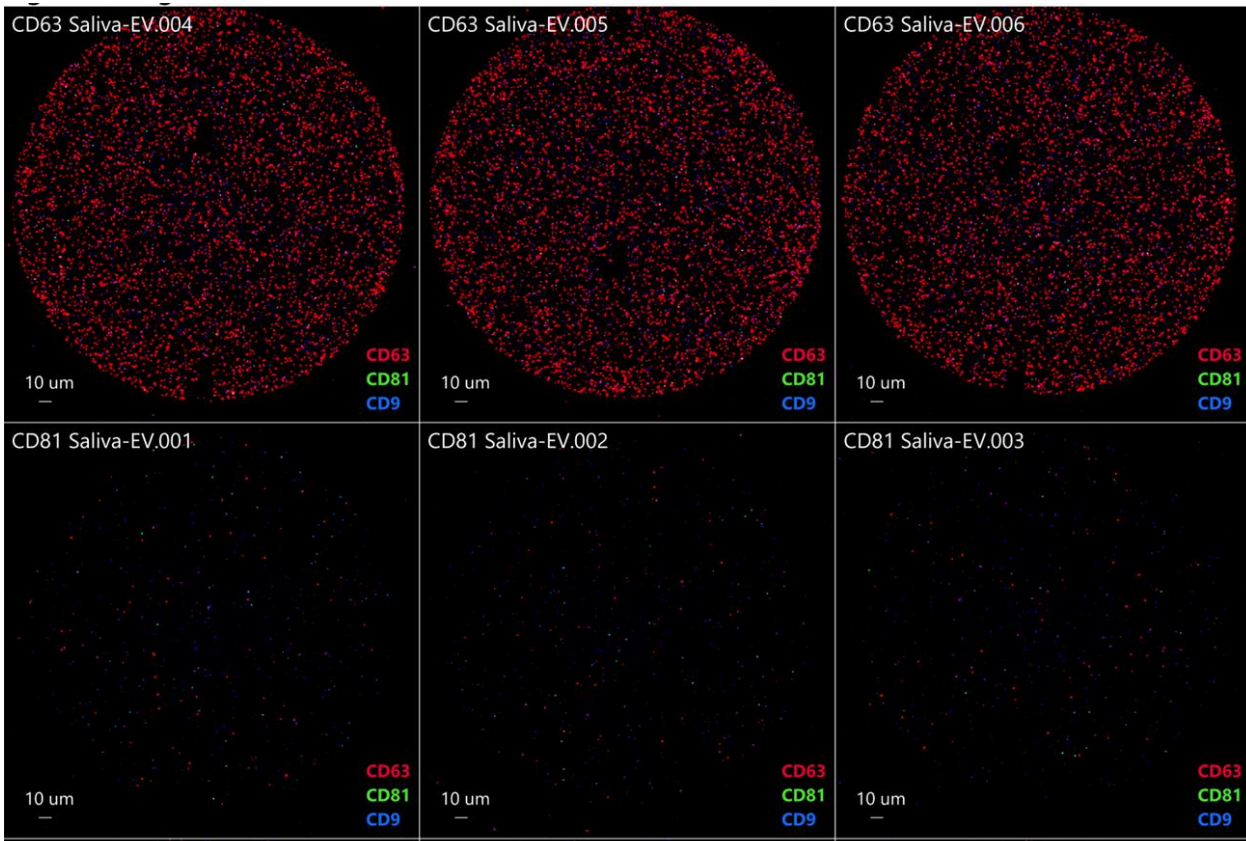
Supplementary Figure S1. Dynamic light scattering (DLS) intensity-weighted size distributions of salivary extracellular vesicles. (a) Representative DLS profile showing a multimodal size distribution with peaks at ~8.8 nm, ~28.8 nm, and ~203.4 nm, with a Z-average diameter of ~99 nm and a polydispersity index (PDI) of 0.265, indicating moderate heterogeneity. (b) Representative DLS profile from a distinct sample exhibiting a dominant population at ~166.6 nm and a secondary peak at ~13.7 nm, with a Z-average diameter of ~507 nm and higher polydispersity (PDI = 0.540), reflecting the presence of larger particles or aggregates. Intensity-weighted distributions emphasize larger vesicles due to scattering bias.



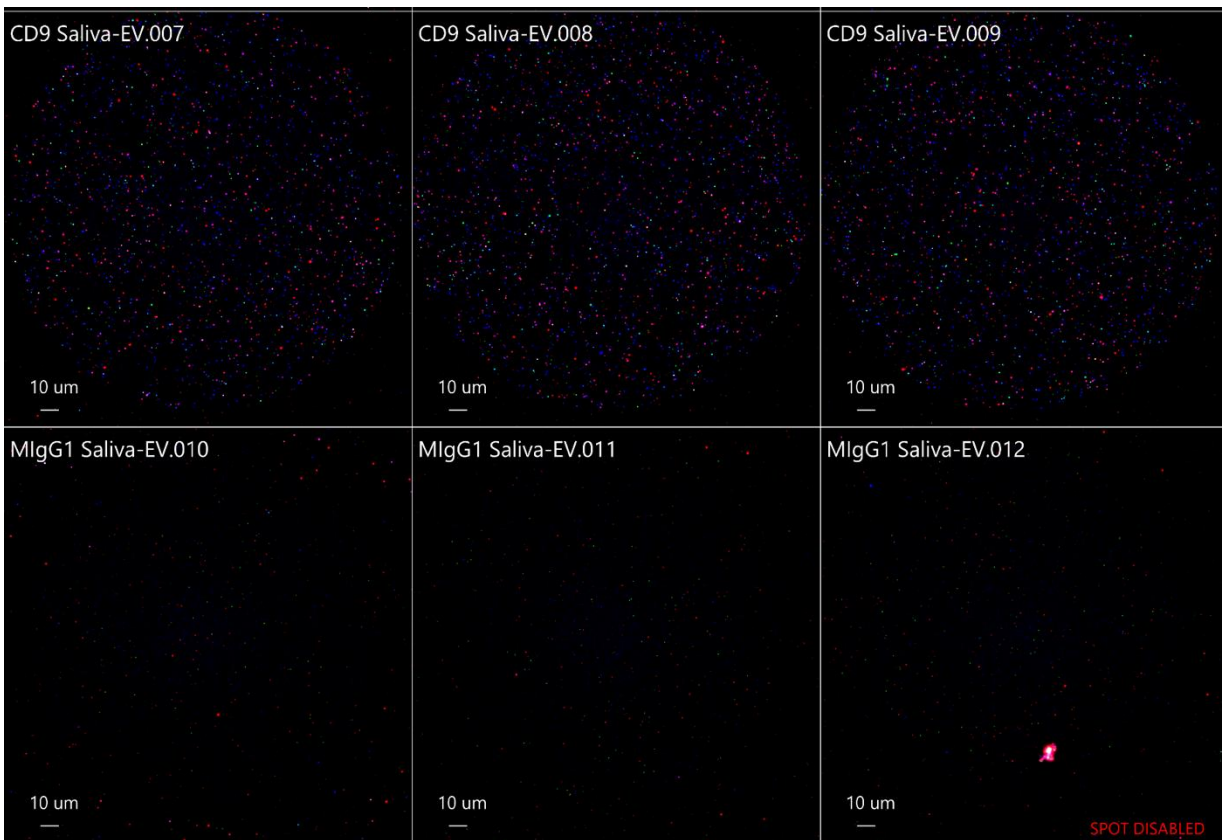


Supplementary Figure S2. Nanoparticle tracking analysis (NTA) size distribution profiles of salivary extracellular vesicles. (a) Representative NTA size distribution showing a predominant EV population centered at ~150 nm (mean ~152.4 nm; mode ~153.8 nm), with particle concentration of $\sim 4.49 \times 10^{10}$ particles/mL and a distribution spanning ~100–250 nm. (b) Representative NTA profile from a distinct sample demonstrating a dominant population at smaller diameters (mean ~115.2 nm; mode ~91.8 nm), with particle concentration of $\sim 3.97 \times 10^{11}$ particles/mL. Distribution percentiles (D10, D50, D90) confirm that the majority of particles fall within the expected size range for small extracellular vesicles. These results highlight inter-sample variability while maintaining a consistent nanoscale EV population characteristic of exosome-enriched fractions.

a



b



Supplementary Figure S3. Representative ExoView interferometric and fluorescence images of salivary extracellular vesicles across capture antibodies and controls. (a) Representative ExoView images of EVs captured using anti-CD63 and anti-CD81 antibodies from independent samples (n = 3 per condition). CD63 capture surfaces show dense particle binding with predominant CD63 signal (red), whereas CD81 capture surfaces exhibit lower particle density with detectable CD81 (green) and co-localized signals, reflecting variability in marker expression across EV subpopulations. (b) Representative images of EVs captured using anti-CD9 antibodies and IgG1 isotype control. CD9 capture surfaces display moderate particle binding with mixed fluorescence signals (blue), while IgG1 controls show minimal non-specific binding, confirming assay specificity. Fluorescence channels correspond to CD63 (red), CD81 (green), and CD9 (blue). Scale bars, 10 μm .

Channels

R: CD63

G: CD81

B: CD9

● R

● G

● B

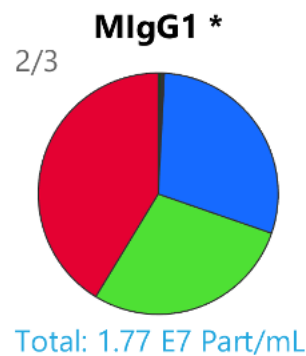
● R/G

● R/B

● G/B

● R/G/B

*Not to Scale



Supplementary Figure S4. Isotype control analysis of non-specific binding in ExoView measurements. Representative colocalization profile obtained from IgG1 isotype control capture surfaces, demonstrating minimal non-specific binding across fluorescence channels. Pie chart representation shows the distribution of detected signals corresponding to CD63 (red), CD81 (green), and CD9 (blue), with negligible co-localization events. The low particle count (total $\sim 1.77 \times 10^7$ particles/mL) and absence of structured marker-specific patterns confirm the specificity of antibody-based EV capture in experimental conditions. Fluorescence channel assignments are indicated, and proportions are not to scale.