

Supplementary Figure 1: Dose dependent titration of cell membrane dye shows Huh 7 cell culture-derived EV concentrations in Fluorescence Mode (FM) and Light Scatter Mode (LSM).

Α

Β



Supplementary Figure 2: PE-labeled fluorescent antibody validation studies in serums of patients with cirrhosis and HCC for quantification of HCC exosomes. (**A**). Shows normalized fluorescent particle concentrations in different dilutions of GP3-PE antibody. (**B**). Shows normalized fluorescent particle concentrations in different dilutions of AFP-PE antibody. GPC3:Glypican 3, AFP: Alpha Fetoprotein. Statistical significance levels were presented as ^{ns} for non-significant, ** for p<0.01, *** for p<0.001

Supplementary Table 1. Optimal cut-offs of F-NTA measurements in serum sample cohort for diagnosing HCC cases

Variable	Cut off (particle/ml)*	Sensitivity (%)	Specificity (%)
GPC3	8.02 x 10 ⁵	85	65
AFP	2.35 x 10 ⁵	80	60
GPC3+AFP	3.22 x 10 ⁵	95	45

*Cut-off values were normalized by using total and fluorescent particle concentrations.