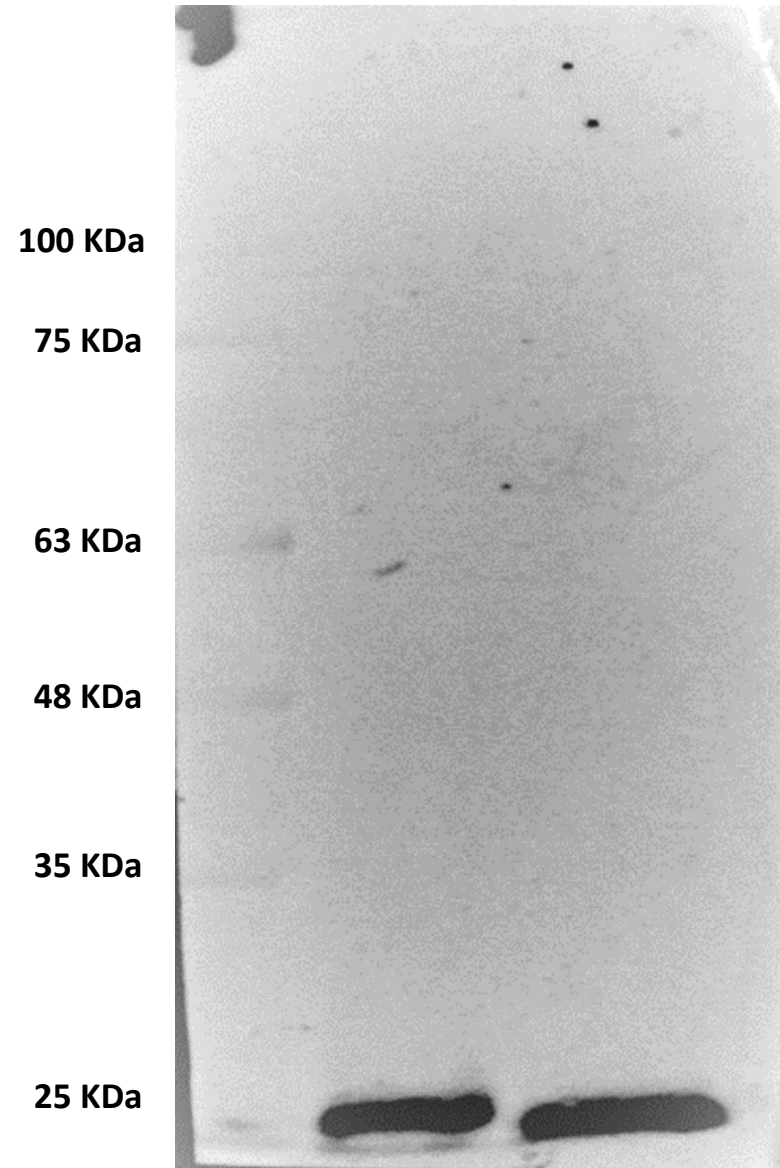
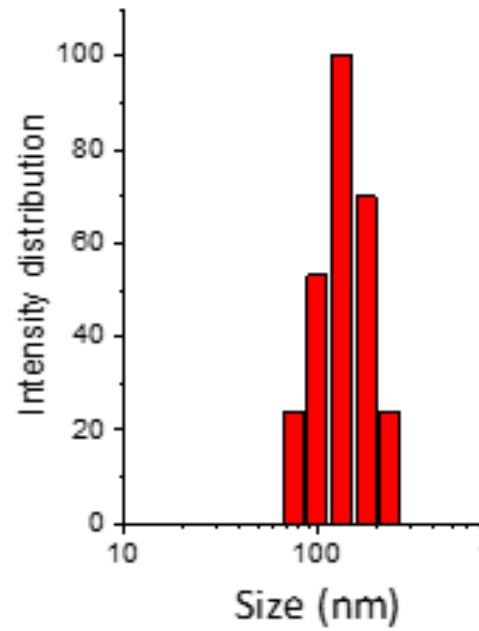




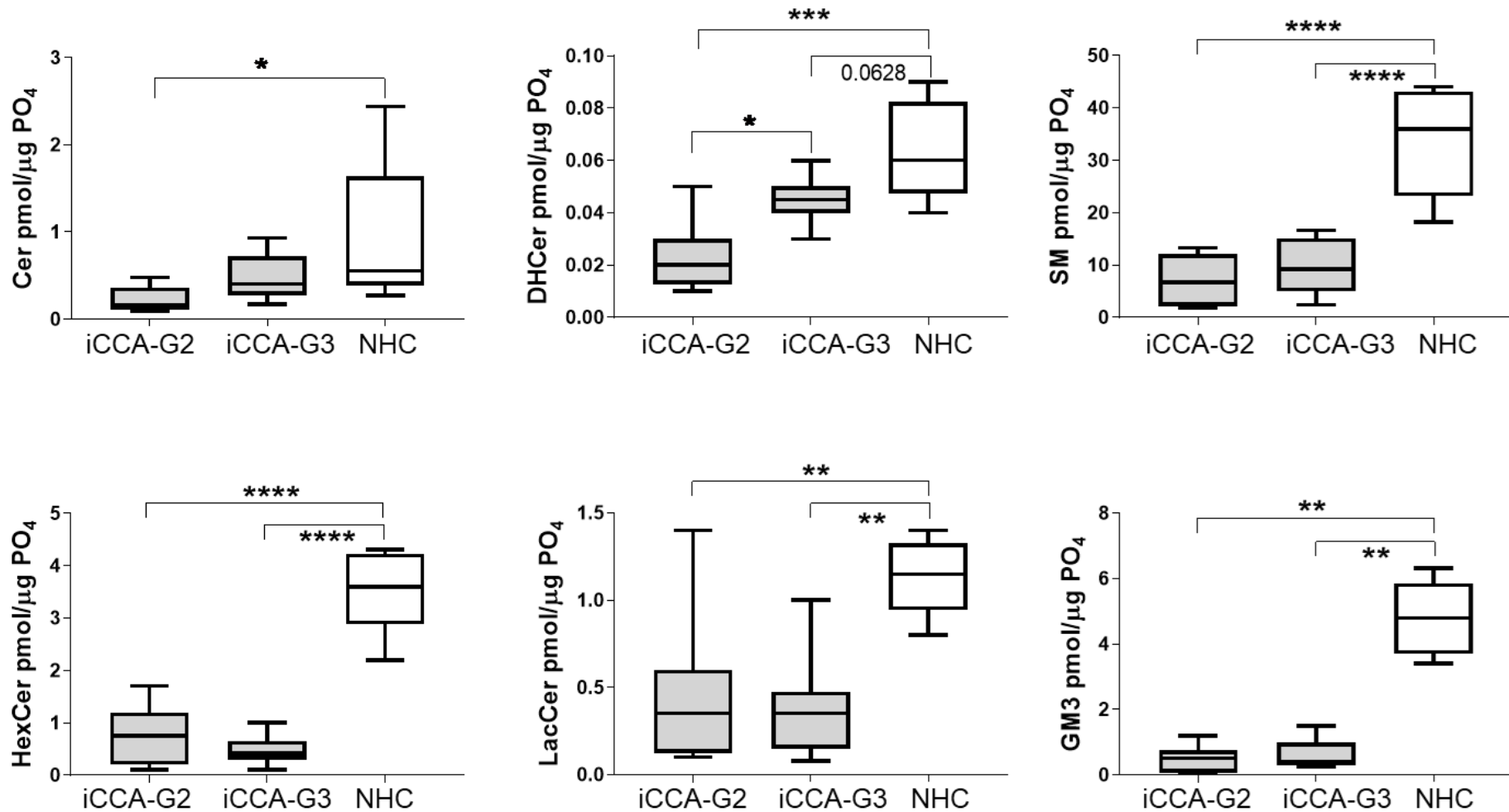
iCCA NHC



**Supplementary Figure 2.** Representative image showing a Western blot of CD81 (lane 1= standard MW ladders; lane 2= primary iCCA-derived EVs; lane 3= NHC-derived EVs). CD81 molecular weight is approximately 25 KDa. Anti-CD81, D502Q, #10037, Cell Signaling, 1:1000 was used.

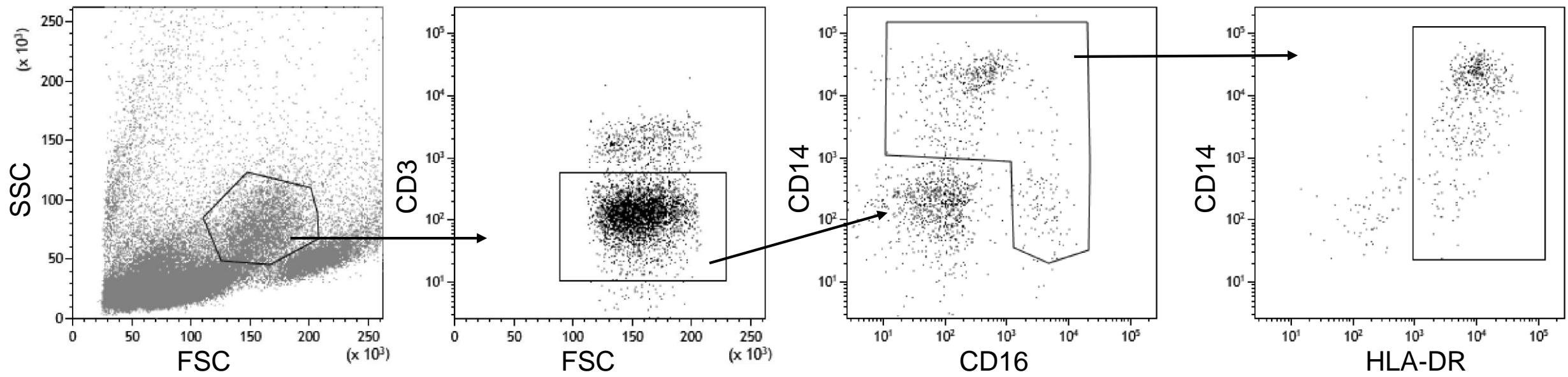


**Supplementary Figure 3.** A representative image of the size distribution of iCCA-derived EVs as assessed by dynamic light scattering, DLS (Hydrodynamic diameter, intensity weighted).

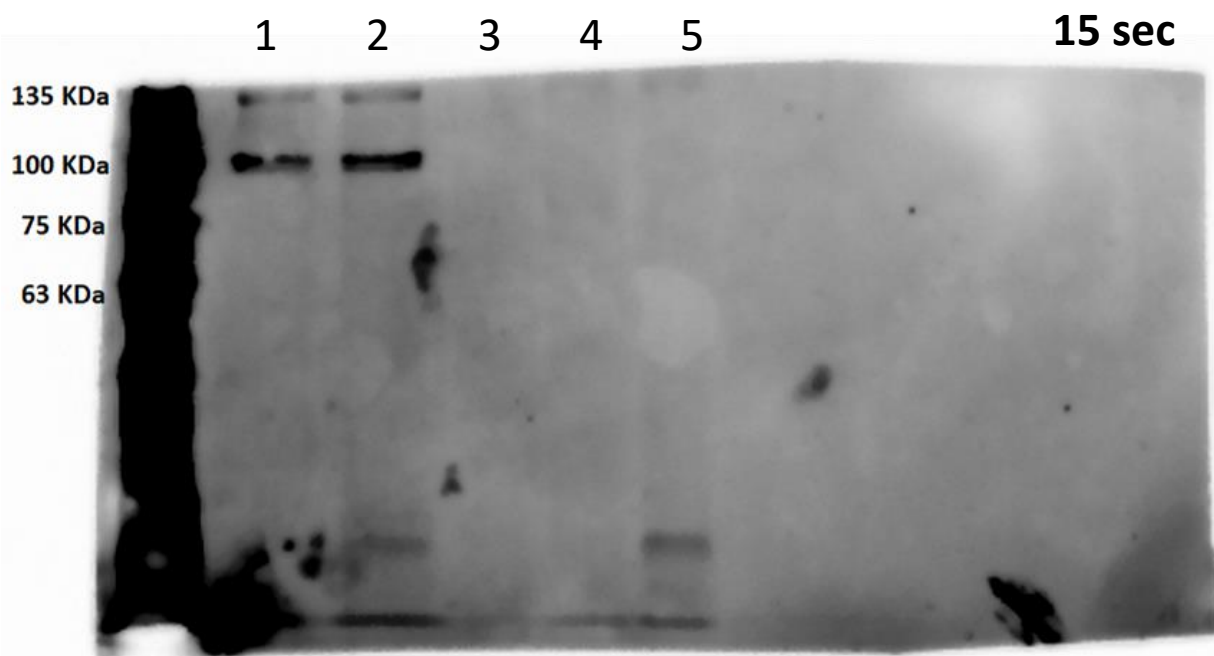
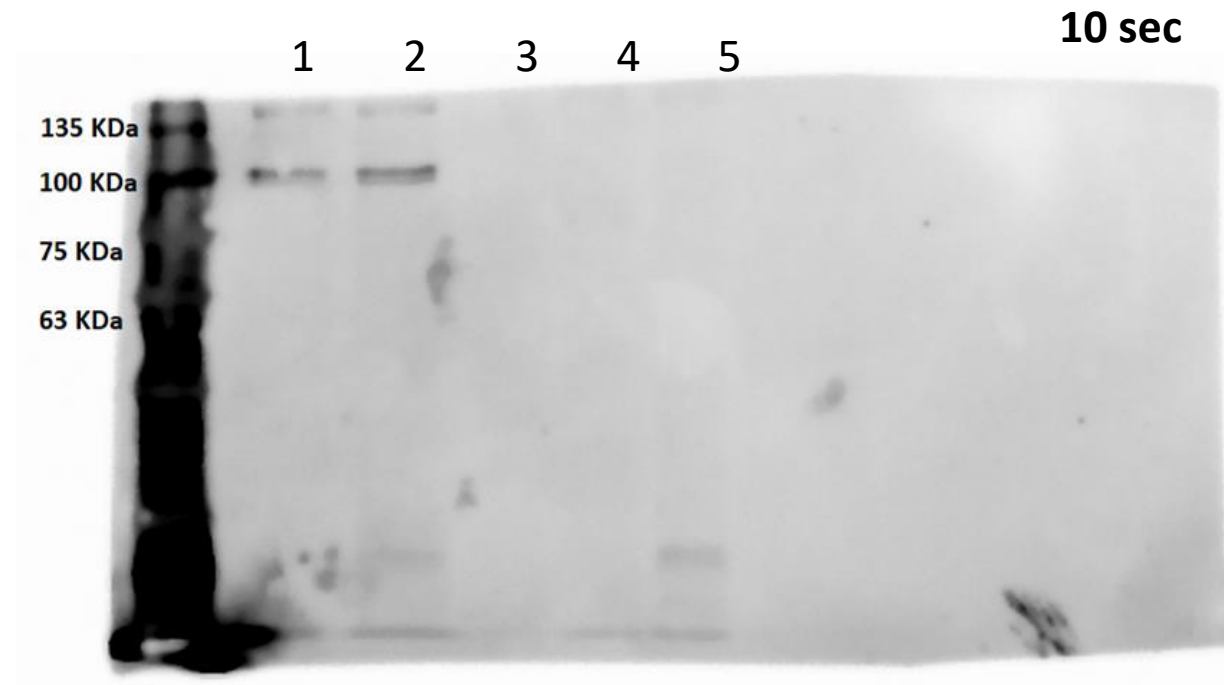
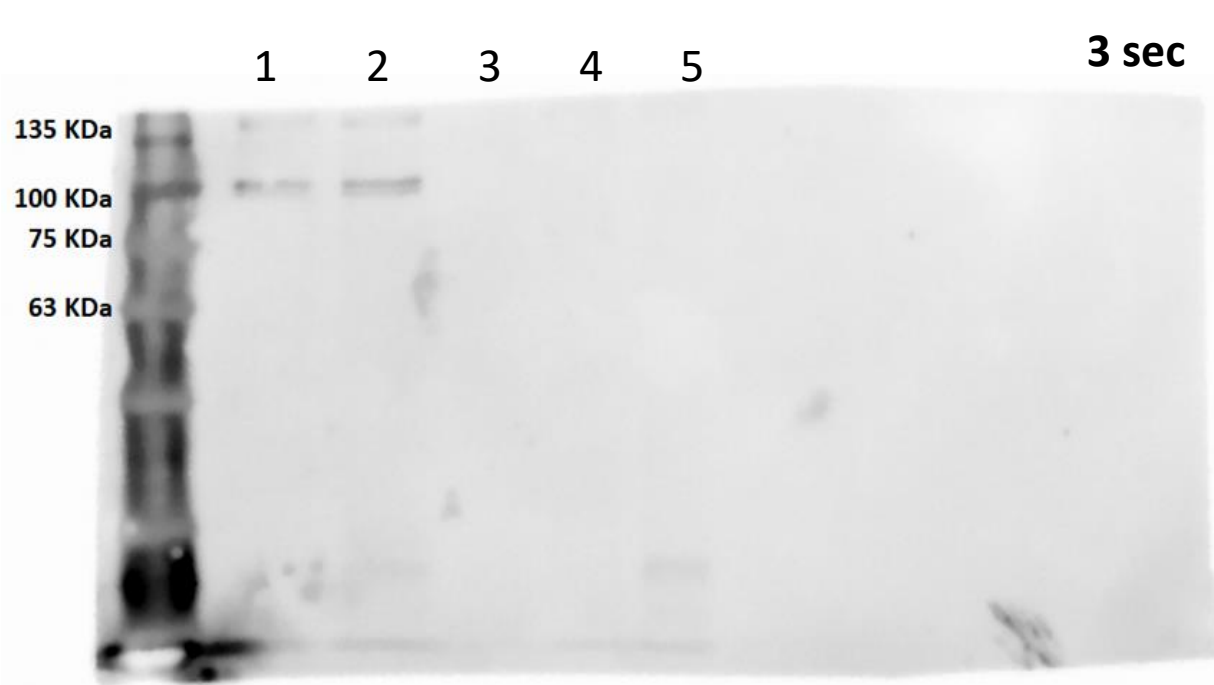


**Supplementary Figure 4.** EV-SPL expression in iCCA-G2, iCCA-G3 and NHC. iCCA samples were stratified according to tumor grade. G2, moderately-differentiated tumors (n = 8); G3, poorly-differentiated tumors (n = 8). Middle bars represent median values; box plots are 25% and 75% percentiles; whiskers are minimum and maximum values.

\*p <0.05, \*\*p <0.01, \*\*\*p <0.001, \*\*\*\*p <0.0001. The one-way ANOVA and Tukey's multiple comparisons tests were used to compare data except for GM3 in which Dunn's multiple comparisons test was used.



**Supplementary Figure 5.** Gating strategy adopted for the experiments presented in figure 3.



**Supplementary Figure 6.** Original, uncropped blots shown in figure 1 at multiple exposures (3, 10 and 15 seconds). lane 1= 15 µg protein extract from primary iCCA cell line; lane 2= 15 µ g protein extract from NHC; lanes 3-4 and 5= 5 µg protein extract from primary iCCA cell lines.