

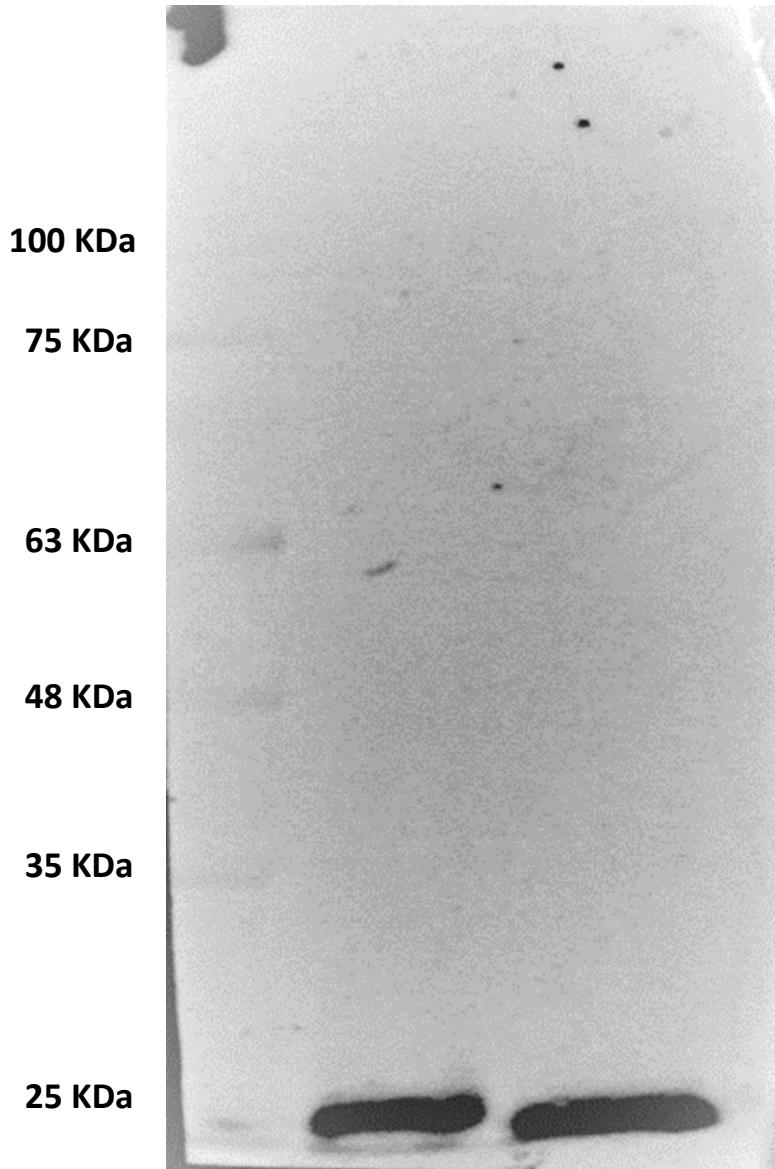
**b**

| Patients                   | #iCCA1 | #iCCA2 | #iCCA3 | #iCCA4 | #iCCA5 | #iCCA6 | #iCCA7 |
|----------------------------|--------|--------|--------|--------|--------|--------|--------|
| Male (M), Female (F)       | M      | M      | F      | M      | M      | M      | M      |
| Age (years)                | 71     | 74     | 73     | 76     | 81     | 81     | 65     |
| ALT (mU/ml)                | 27     | 38     | 16     | 17     | 27     | 11     | 19     |
| AST (mU/ml)                | 26     | 27     | 21     | 25     | 32     | 17     | 24     |
| PLT ( $10^3/\mu\text{l}$ ) | 234    | 287    | 233    | 180    | 191    | 233    | 304    |
| Glucose (mg/dl)            | 101    | 163    | 94     | 140    | 120    | 108    | 97     |
| TG (mg/dl)                 | 204    | 82     | 70     | NA     | 168    | 83     | 124    |
| CHOL (mg/dl)               | 185    | 228    | 132    | NA     | 219    | 324    | 129    |
| HDL (mg/dl)                | 35     | 70     | 59     | NA     | 53     | 54     | 35     |
| LDL (mg/dl)                | 109    | 141    | 59     | NA     | 132    | 253    | 80     |
| Alb (g/dl)                 | 4,2    | 42,9   | 4,1    | 4      | 4,6    | 42,6   | 4      |
| Tot Bil (mg/dl)            | 0,8    | 0,7    | 0,9    | 1,24   | 1,9    | 0,4    | 0,57   |
| CA 19.9 (IU/ml)            | 116    | 51,9   | 10,2   | 4,8    | 32,3   | 353,3  | 7,7    |
| BMI                        | 28,34  | 27,76  | 24,91  | 32,9   | 24,22  | 31,05  | NA     |
| DM2                        | Y      | Y      | N      | Y      | NA     | N      | Y      |
| Dyslipidemia               | N      | Y      | N      | N      | Y      | Y      | N      |
| Cirrhosis                  | N      | N      | N      | N      | N      | N      | N      |
| Vascular Invasion          | N      | Y      | N      | N      | Y      | N      | Y      |
| Tumor size (cm)            | 3,6    | >5     | <2     | 5      | 2,2    | 3,7    | >5     |
| Tumor Grading              | G2     | G2     | G2     | G3     | G2     | G3     | G3     |
| Tumor Stage                | T3     | T2     | T2     | T2     | T1     | T1     | T2     |
| Etiology                   | NA     | NASH   | NASH   | NASH   | NASH   | NA     | HBV    |

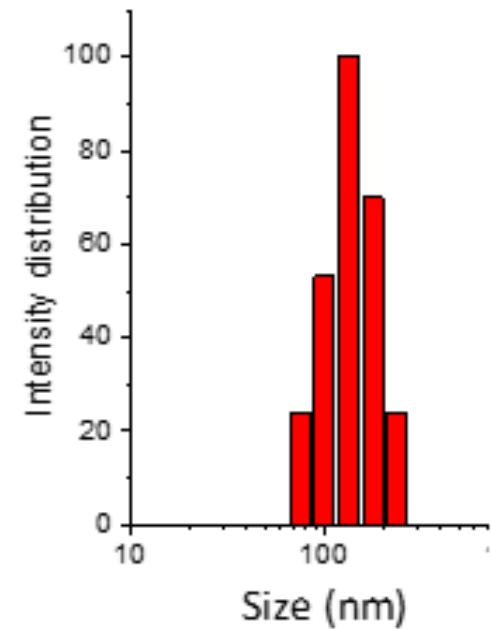
ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet; TG, triglyceride; CHOL, cholesterol; HDL, high-density lipoprotein; LDL, low-density Lipoprotein; Alb, albumin; Tot Bil, total bilirubine; CA19.9, carbohydrate antigen 19-9; BMI, body mass index; DM2, type 2 diabetes mellitus; HBV, hepatitis B virus; HCV, Hepatitis C virus; NASH, nonalcoholic steatohepatitis; Y, Yes; N, No; NA, not available.

**Supplementary figure 1.** (a) Cytokeratin 19 (CK19) expression on representative iCCA patients-derived cell cultures. (b) Clinical characteristics of patients from whom cell cultures tested for CK-19 expression were obtained.

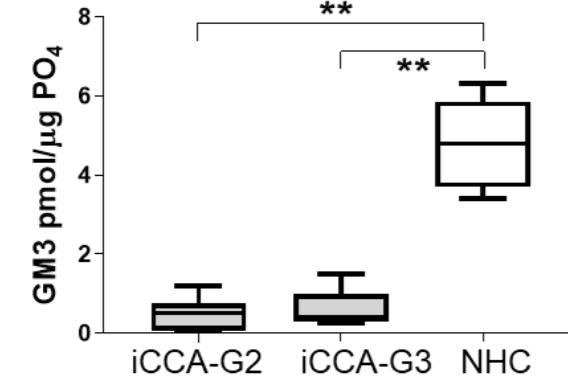
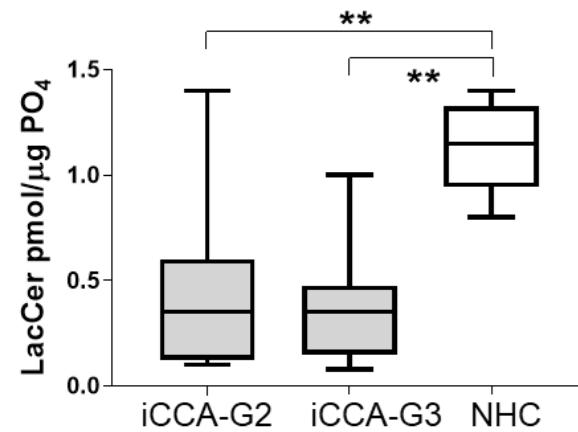
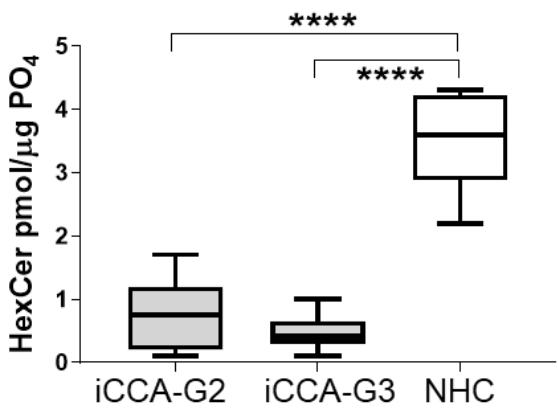
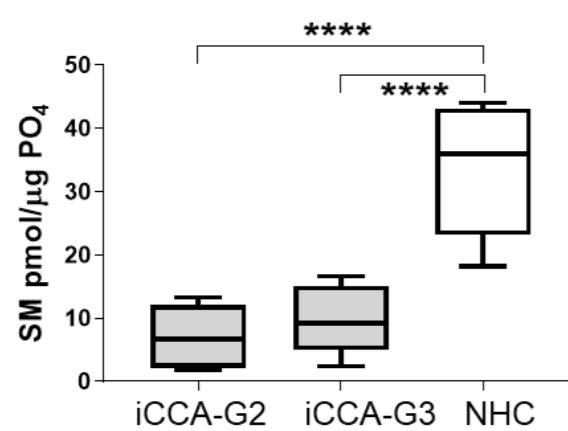
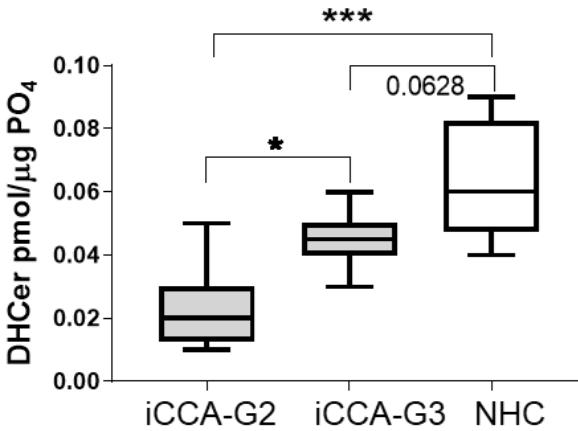
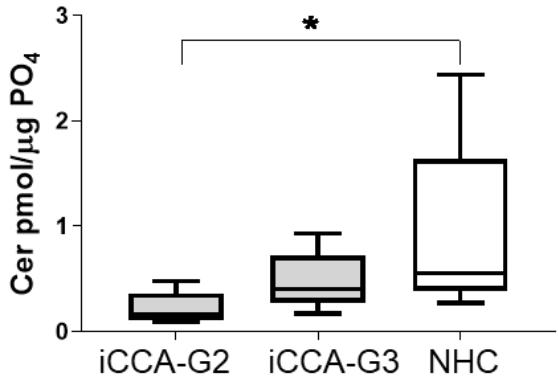
iCCA NHC



**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.

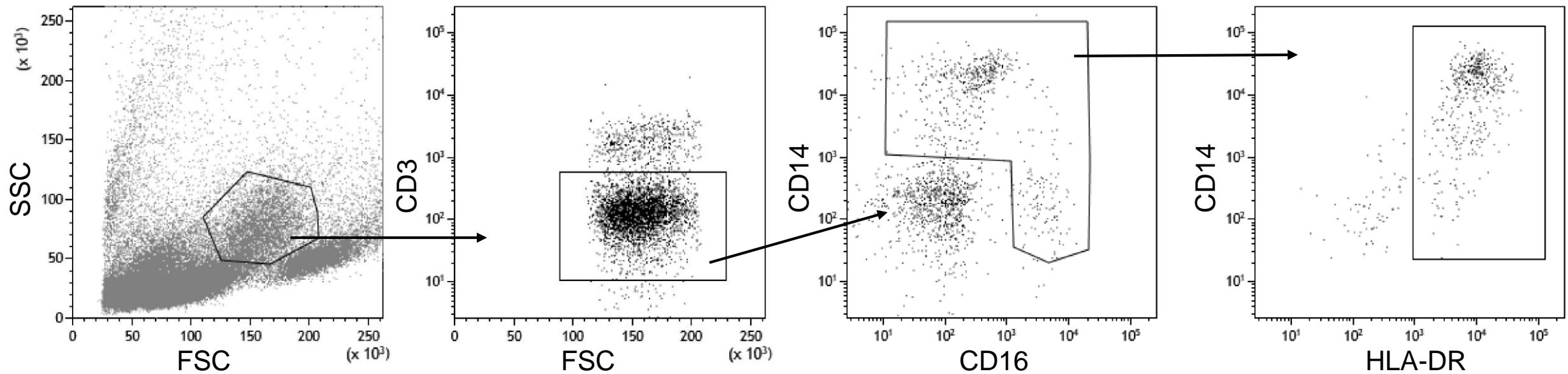


**Supplementary Figure 3.** A representative image of the size distribution of iCCA-derived EVs as assessed by dynamic light scattering, DLS (Hydrodynamic diameter, intensity weighted).

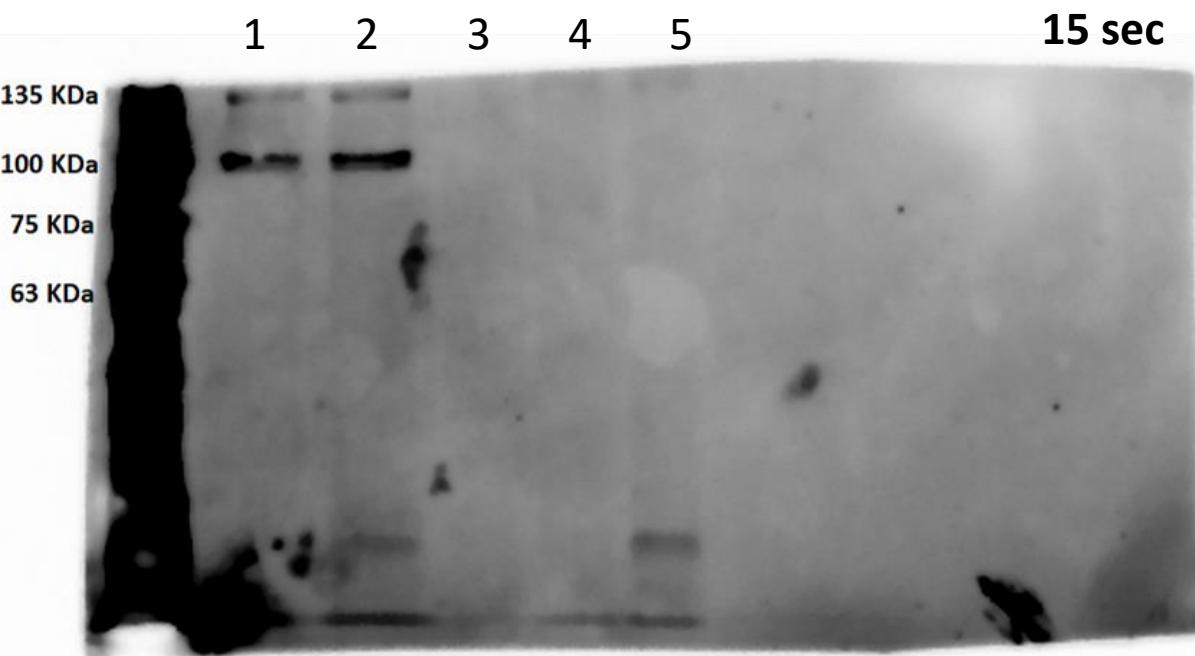
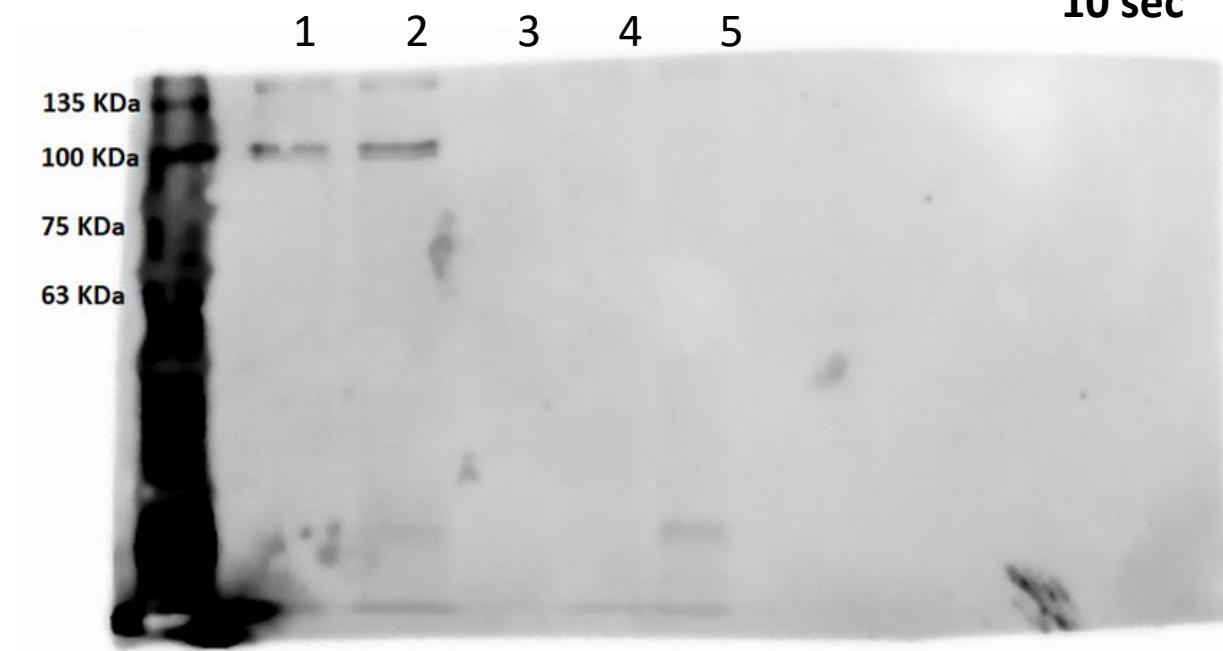
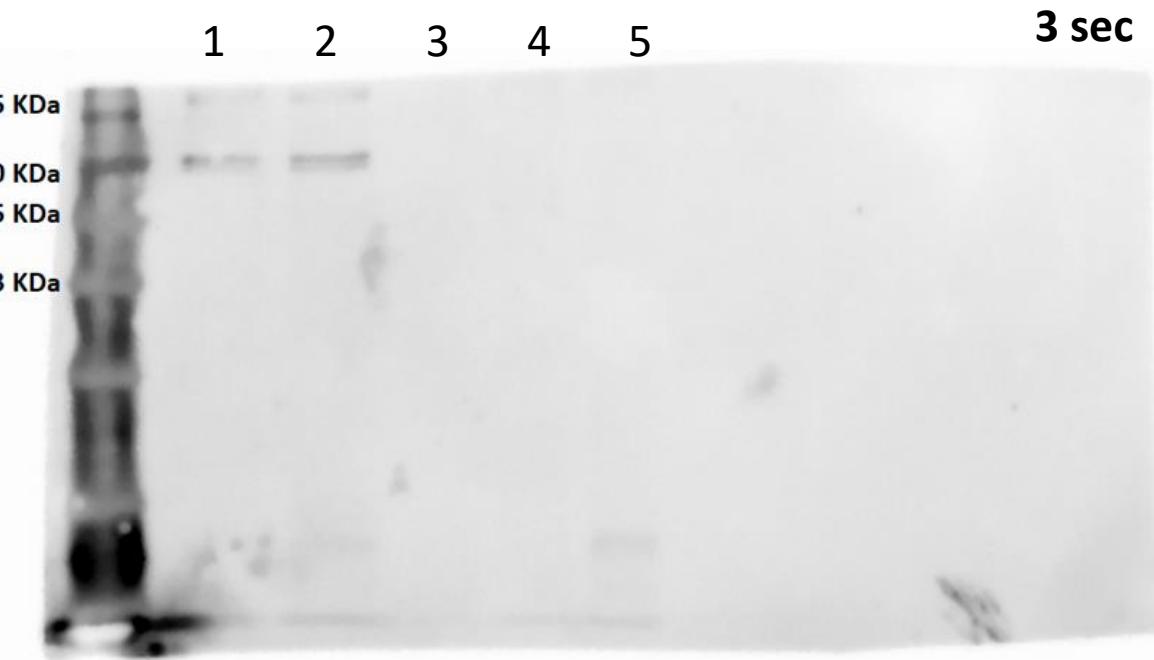


**Supplementary Figure 4.** EV-SPL expression in iCCA-G2, iCCA-G3 and NHC. iCCA samples were stratified according to tumor grade. G2, moderately-differentiated tumors ( $n = 8$ ); G3, poorly-differentiated tumors ( $n = 8$ ). Middle bars represent median values; box plots are 25% and 75% percentiles;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