

Figure S1. Melting curves of the candidate reference genes *ACTB*, *GAPDH*, *HMBS*, *PPIA* and *RPLP0*. (A) PCR amplification efficiencies of the five candidate genes. (B) Annealing temperatures and mean Cq values of the five candidate genes.

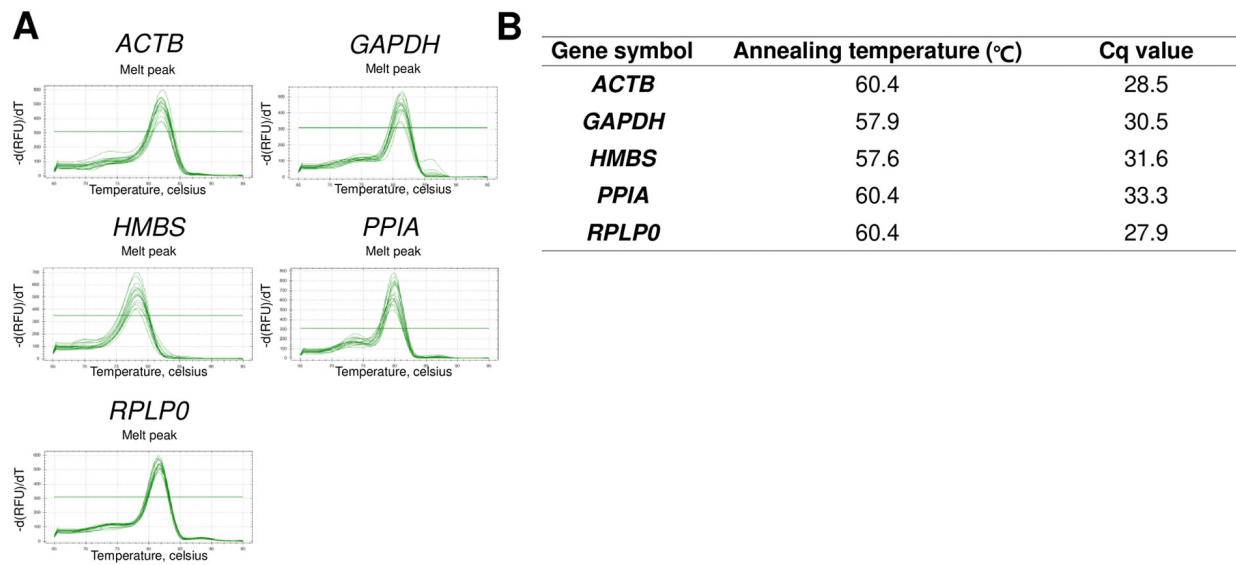


Figure S2. Characterization of serum-derived exosomes. (A) Transmission electron microscopy image showing the spherical morphology of isolated exosomes; scale bar, 100 nm. (B) Immunoblots showing EV marker expression in the isolated exosomes. Huh-7 cell lysate was used as a control. EV marker, extracellular vesicle marker; ER marker, endoplasmic reticulum marker.

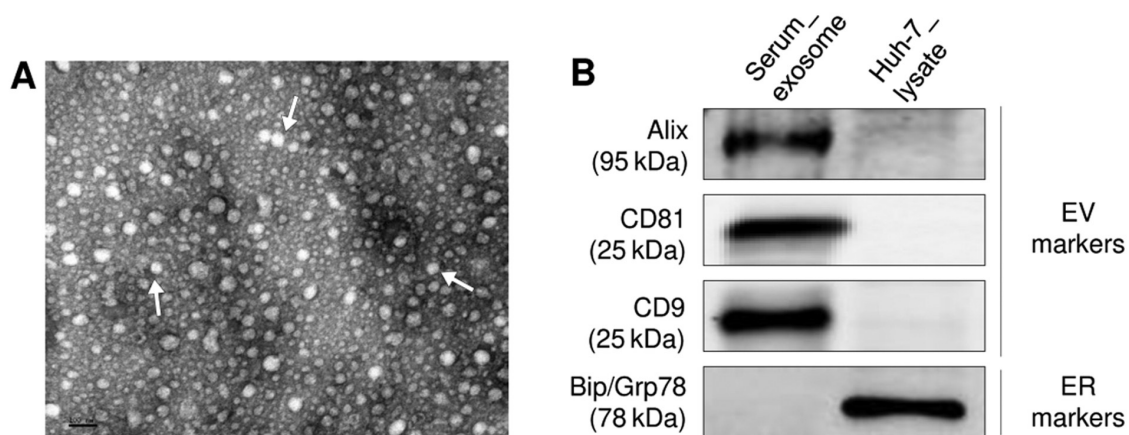


Table SI. Patient clinical characteristics.

| Characteristic                            | N=40 (%)       |
|-------------------------------------------|----------------|
| Age, years                                | 56.43 (9.26)   |
| Male sex                                  | 34 (85)        |
| Etiology                                  |                |
| Hepatitis C virus infection               | 3 (11.11)      |
| Cirrhosis                                 | 19 (47.5)      |
| Differentiation                           |                |
| I-II                                      | 11 (27.5)      |
| III-IV                                    | 21 (52.5)      |
| Platelet count, $\times 10^3/\mu\text{l}$ | 188.35 (79.37) |
| Albumin, g/dl                             | 4.35 (0.63)    |
| Total bilirubin, mg/dl                    | 0.74 (0.86)    |
| Creatinine, mg/dl                         | 0.89 (0.18)    |
| Aspartate transaminase, IU/l              | 55 (80.1)      |
| Alanine aminotransferase, IU/l            | 35.3 (22.41)   |
| AFP (>200 ng/ml)                          | 12 (30.77)     |
| PIVKA-II, (>40 mAU/ml)                    | 27 (79.41)     |
| Vascular invasion                         | 20 (74.07)     |
| Barcelona Clinic Liver Cancer stage       |                |
| 0                                         | 7 (17.5)       |
| A                                         | 13 (32.5)      |
| B                                         | 3 (7.5)        |
| C                                         | 16 (40)        |
| D                                         | 1 (2.5)        |
| mUICC stage                               |                |
| I                                         | 7 (17.5)       |
| II                                        | 13 (32.5)      |
| III                                       | 13 (32.5)      |
| IVA                                       | 4 (10)         |
| IVB                                       | 3 (7.5)        |

Table SII. Log<sub>2</sub> fold change and P-value for expression levels of 14 reference genes selected between non-tumor group and hepatocellular carcinoma groups using the GSE114564 database.

| Gene          | Fold change |              |             |              |             |              |
|---------------|-------------|--------------|-------------|--------------|-------------|--------------|
|               | NL vs. eHCC | NL vs. avHCC | CH vs. eHCC | CH vs. avHCC | LC vs. eHCC | LC vs. avHCC |
| <i>ACTB</i>   | 1.06        | 1.05         | 1.01        | 1.00         | 1.02        | 1.01         |
| <i>B2M</i>    | 1.18        | 0.68         | 1.12        | 0.65         | 0.98        | 0.57         |
| <i>GAPDH</i>  | 1.15        | 1.09         | 0.90        | 0.85         | 0.79        | 0.75         |
| <i>GUSB</i>   | 1.07        | 0.69         | 1.14        | 0.74         | 1.03        | 0.67         |
| <i>HMBS</i>   | 1.07        | 1.11         | 0.93        | 0.97         | 0.94        | 0.98         |
| <i>HPRT1</i>  | 0.68        | 0.82         | 0.96        | 1.15         | 1.17        | 1.41         |
| <i>PGK1</i>   | 1.17        | 1.13         | 1.38        | 1.33         | 1.35        | 1.29         |
| <i>PPIA</i>   | 1.15        | 1.14         | 1.08        | 1.08         | 1.03        | 1.02         |
| <i>RPL13A</i> | 0.72        | 0.73         | 0.64        | 0.65         | 0.62        | 0.63         |
| <i>RPLP0</i>  | 0.89        | 1.02         | 0.93        | 1.07         | 0.89        | 1.03         |
| <i>SDHA</i>   | 0.79        | 0.47         | 0.99        | 0.59         | 1.23        | 0.74         |
| <i>TBP</i>    | 0.95        | 0.96         | 1.00        | 1.00         | 1.12        | 1.13         |
| <i>TFRC</i>   | 0.99        | 1.06         | 1.56        | 1.67         | 1.69        | 1.80         |
| <i>YWHAZ</i>  | 1.42        | 1.75         | 1.42        | 1.75         | 1.37        | 1.69         |

| Gene          | P-value     |              |             |              |             |              |
|---------------|-------------|--------------|-------------|--------------|-------------|--------------|
|               | NL vs. eHCC | NL vs. avHCC | CH vs. eHCC | CH vs. avHCC | LC vs. eHCC | LC vs. avHCC |
| <i>ACTB</i>   | 0.9999      | 0.9999       | 0.9999      | 0.9999       | 0.9999      | 0.9999       |
| <i>B2M</i>    | 0.9999      | 0.3582       | 0.9999      | 0.1185       | 0.9999      | 0.0067       |
| <i>GAPDH</i>  | 0.9999      | 0.9999       | 0.9999      | 0.9999       | 0.4575      | 0.0969       |
| <i>GUSB</i>   | 0.9999      | 0.0061       | 0.8168      | 0.1843       | 0.9999      | 0.0301       |
| <i>HMBS</i>   | 0.9999      | 0.9999       | 0.9999      | 0.9999       | 0.9999      | 0.9999       |
| <i>HPRT1</i>  | 0.0095      | 0.1982       | 0.9999      | 0.9999       | 0.9999      | 0.1178       |
| <i>PGK1</i>   | 0.9507      | 0.9999       | 0.0004      | 0.0255       | 0.0326      | 0.6777       |
| <i>PPIA</i>   | 0.4854      | 0.5129       | 0.9999      | 0.9999       | 0.9999      | 0.9999       |
| <i>RPL13A</i> | 0.0687      | 0.4164       | 0.0002      | 0.0008       | 0.0023      | 0.0141       |
| <i>RPLP0</i>  | 0.9999      | 0.9999       | 0.9999      | 0.9999       | 0.9999      | 0.9999       |
| <i>SDHA</i>   | 0.6492      | 0.0001       | 0.9999      | 0.0001       | 0.9999      | 0.2327       |
| <i>TBP</i>    | 0.9999      | 0.9999       | 0.9999      | 0.9999       | 0.5414      | 0.1175       |
| <i>TFRC</i>   | 0.9999      | 0.9999       | 0.0270      | 0.0005       | 0.0494      | 0.0044       |
| <i>YWHAZ</i>  | 0.0051      | 0.0001       | 0.0024      | 0.0001       | 0.0610      | 0.0058       |

NL, Normal; eHCC, early hepatocellular carcinoma; avHCC, advanced hepatocellular carcinoma.