

Supplementary Table S1. Baseline characteristics of the study participants

|                                                                         | Discovery cohort | Validation cohort |
|-------------------------------------------------------------------------|------------------|-------------------|
|                                                                         | iCCA (n=15)      |                   |
| Age (mean $\pm$ SD)                                                     | 67 $\pm$ 8.2     | 65.1 $\pm$ 8.7    |
| Age [range]                                                             | 52-78            | 51-84             |
| <b>Gender, n (%)</b>                                                    |                  |                   |
| Males                                                                   | 8 (53.3)         | 8 (53.33)         |
| Females                                                                 | 7 (46.6)         | 7 (46.67)         |
| <b>Environment, n (%)</b>                                               |                  |                   |
| Urban                                                                   | 6 (40)           | 10 (66.67)        |
| Rural                                                                   | 9 (60)           | 5 (33.33)         |
| <b>Underlying disease, n (%)</b>                                        |                  |                   |
| Hepatitis B virus                                                       | 1 (6.6)          | 2 (13.33)         |
| Hepatitis C virus                                                       |                  |                   |
| Alcohol abuse                                                           | 2 (13.3)         | 3 (20)            |
| Diabetes                                                                | 3 (20)           | 2 (13.33)         |
| Autoimmune                                                              |                  | 1 (6.6)           |
| No                                                                      | 9 (60)           | 7 (46.6)          |
| <b>Nodules, n (%)</b>                                                   |                  |                   |
| Single                                                                  | 0 (0)            | 1 (6.67)          |
| Two or more                                                             | 3 (20)           | 5 (33.33)         |
| Not available                                                           | 12 (80)          | 9 (60)            |
| <b>Nodule size, n (%)</b>                                               |                  |                   |
| $\leq 3$ cm                                                             | 1 (6.6)          | 0 (0)             |
| $> 3$ cm                                                                | 8 (53.3)         | 1 (6.67)          |
| Not available                                                           | 6 (40)           | 14 (93.33)        |
| <b>Child-Pugh score, n (%)</b>                                          |                  |                   |
| A                                                                       |                  | 1 (6.67)          |
| B                                                                       |                  | 2 (13.33)         |
| C                                                                       |                  |                   |
| Not available                                                           | 15 (100)         | 12 (80)           |
| <b>METAVIR score, n(%)</b>                                              |                  |                   |
| F0                                                                      |                  |                   |
| F1                                                                      |                  |                   |
| F2                                                                      |                  |                   |
| F3                                                                      |                  |                   |
| F4                                                                      |                  | 1 (6.67)          |
| Not available                                                           | 15 (100)         | 14 (93.33)        |
| <b>Laboratory [normal values]<br/>(units), mean <math>\pm</math> SD</b> |                  |                   |
| Albumin [3.5 - 5.2] (g/dL)                                              | 3.5 $\pm$ 0.4    | 3.63 $\pm$ 0.69   |

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|                                                                | Discovery cohort     | Validation cohort      |
|----------------------------------------------------------------|----------------------|------------------------|
|                                                                | iCCA (n=15)          |                        |
| <b>Laboratory [normal values]<br/>(units), median [Q1; Q3]</b> |                      |                        |
| AFP [0.9-7.0] (ng/mL)                                          | 3.9 [2.5; 35.85]     | 6.11 [2.82; 101]       |
| CA 19-9 [2-34] (U/mL)                                          | 117.9 [13.5; 158.15] | 30 [5; 300]            |
| CEA [0.3-4.7] (ng/mL)                                          | 5.1 [2.4; 14.3]      | 2.63 [1.51; 8.86]      |
| AST [10-50] (U/I)                                              | 60.5 [27.75; 80.5]   | 83 [43.5 ; 152.5]      |
| ALT [10-50] (U/I)                                              | 29 [23.75; 108.25]   | 54 [22; 135]           |
| γGT [8-61] (U/I)                                               | 128.5 [77.5; 505.25] | 195 [162; 369]         |
| AP [40-129] (U/I)                                              | 478 [228.25; 937.5]  | 280 [189.5; 499]       |
| TB [0.1-1.2] (mg/dL)                                           | 1.25 [0.8; 4.4]      | 2.8 [0.85; 8]          |
| PT [11.8-14.9] (sec)                                           | 19.2 [17.93; 24.18]  | 16.1 [14.85; 18.4]     |
| INR [0.8-1.2]                                                  | 1.15 [1.08; 1.47]    | 1.19 [1.07; 1.3]       |
| Glucose [82-115] (mg/dL)                                       | 112 [94.3; 121.1]    | 90 [80; 100.5]         |
| Triglycerides [50-165] (mg/dL)                                 | 90.5 [81.75; 99.25]  | 124 [78.5; 170]        |
| CRP [0-0.5] (mg/dL)                                            | 2.91 [0.6; 6.38]     | 2.4 [1.17; 5.52]       |
| Cholesterol [110-220] (mg/dL)                                  | 166 [165.5; 190.25]  | 175.5 [119.75; 198.25] |
|                                                                | HCC (n=15)           |                        |
| Age (mean ± SD)                                                | 64.4 ± 8.6           | 65.33 ± 7.29           |
| Age [range]                                                    | 50-81                | 55-82                  |
| <b>Gender, n (%)</b>                                           |                      |                        |
| Males                                                          | 12 (80)              | 7 (46.67)              |
| Females                                                        | 3 (20)               | 8 (53.33)              |
| <b>Environment, n (%)</b>                                      |                      |                        |
| Urban                                                          | 7 (46.6)             | 11 (73.33)             |
| Rural                                                          | 8 (53.3)             | 4 (26.67)              |
| <b>Underlying disease, n (%)</b>                               |                      |                        |
| Hepatitis B virus                                              | 2 (13.3)             | 5 (33.33)              |
| Hepatitis C virus                                              | 7 (46.6)             | 4 (26.67)              |
| Alcohol abuse                                                  | 3 (20)               | 4 (26.67)              |
| Diabetes                                                       | 2 (13.3)             | 0 (0)                  |
| No                                                             | 3 (20)               | 1 (6.6)                |
| <b>Nodules, n (%)</b>                                          |                      |                        |
| Single                                                         | 4 (26.6)             | 4 (26.67)              |
| Two or more                                                    | 8 (53.3)             | 11 (73.33)             |
| Not available                                                  | 3 (20)               | 0 (0)                  |
| <b>Nodule size, n (%)</b>                                      |                      |                        |
| ≤3 cm                                                          | 6 (40)               | 7 (46.67)              |
| >3 cm                                                          | 7 (46.6)             | 3 (20)                 |
| Not available                                                  | 2 (13.3)             | 5 (33.33)              |

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|                                                                         | Discovery cohort    | Validation cohort   |
|-------------------------------------------------------------------------|---------------------|---------------------|
|                                                                         | HCC (n=15)          |                     |
| <b>Child-Pugh score, n (%)</b>                                          |                     |                     |
| A                                                                       | 9 (60)              | 11 (73.33)          |
| B                                                                       | 2 (13.3)            | 2 (13.33)           |
| C                                                                       | 1 (6.6)             | 2 (13.33)           |
| Not available                                                           | 3 (20)              |                     |
| <b>METAVIR score, n (%)</b>                                             |                     |                     |
| F0                                                                      |                     |                     |
| F1                                                                      |                     |                     |
| F2                                                                      |                     | 1 (6.67)            |
| F3                                                                      |                     |                     |
| F4                                                                      | 15 (100)            | 14 (93.33)          |
| Not available                                                           |                     |                     |
| <b>Laboratory [normal values]<br/>(units), mean <math>\pm</math> SD</b> |                     |                     |
| Albumin [3.5 - 5.2] (g/dL)                                              | 3.4 $\pm$ 0.8       | 3.67 $\pm$ 0.62     |
| <b>Laboratory [normal values]<br/>(units), median [Q1; Q3]</b>          |                     |                     |
| AFP [0.9-7.0] (ng/mL)                                                   | 15 [3.85; 34.85]    | 19 [5.64; 571]      |
| CA 19-9 [2-34] (U/mL)                                                   | 36.5 [24.7; 125.25] | 32.5 [15.95; 51.75] |
| CEA [0.3-4.7] (ng/mL)                                                   | -                   | 1.79 [1.05; 4.13]   |
| AST [10-50] (U/L)                                                       | 48 [29.5; 70]       | 63 [42; 112.5]      |
| ALT [10-50] (U/L)                                                       | 29 [23; 37]         | 51 [25.5; 67]       |
| $\gamma$ GT [8-61] (U/L)                                                | 67 [43; 80]         | 60 [34; 101.5]      |
| AP [40-129] (U/L)                                                       | 286 [262; 443]      | 154 [95; 191.5]     |
| TB [0.1-1.2] (mg/dL)                                                    | 1.3 [0.9; 1.75]     | 1.2 [0.9; 2.2]      |
| PT [11.8-14.9] (sec)                                                    | 20.95 [18.5; 22.47] | 16.4 [15.75; 18.35] |
| INR [0.8-1.2]                                                           | 1.35 [1.14; 1.42]   | 1.26 [1.17; 1.43]   |
| Glucose [82-115] (mg/dL)                                                | 97.7 [88.3; 104]    | 102 [93.25; 116]    |
| Triglycerides [50-165] (mg/dL)                                          | 159 [156; 162]      | 70.5 [59.25; 78]    |
| CRP [0-0.5] (mg/dL)                                                     | 0.43 [0.37; 0.68]   | 0.6 [0.35; 1.27]    |
| Cholesterol [110-220] (mg/dL)                                           | 162 [149; 181]      | 110.5 [92.5; 123]   |
|                                                                         | PSC (n=15)          |                     |
| Age (mean $\pm$ SD)                                                     | 52.6 $\pm$ 14.1     | 50.93 $\pm$ 14.41   |
| Age [range]                                                             | 24-76               | 26-78               |
| <b>Gender, n (%)</b>                                                    |                     |                     |
| Males                                                                   | 5 (33.3)            | 8 (53.33)           |
| Females                                                                 | 10 (66.6)           | 7 (46.67)           |
| <b>Environment, n (%)</b>                                               |                     |                     |
| Urban                                                                   | 9 (60)              | 11 (73.33)          |
| Rural                                                                   | 6 (40)              | 4 (26.67)           |

Supplementary Table S1. Baseline characteristics of the study participants

|                                                                         | Discovery cohort    | Validation cohort    |
|-------------------------------------------------------------------------|---------------------|----------------------|
|                                                                         | PSC (n=15)          |                      |
| <b>Underlying disease, n (%)</b>                                        |                     |                      |
| Hepatitis B virus                                                       | 0 (0)               |                      |
| Hepatitis C virus                                                       | 0 (0)               |                      |
| Alcohol abuse                                                           | 0 (0)               | 1 (6.66)             |
| Diabetes                                                                | 3 (20)              | 2 (13.33)            |
| No                                                                      | 12 (80)             | 12 (80)              |
| <b>Child-Pugh score, n (%)</b>                                          |                     |                      |
| A                                                                       |                     | 2 (13.3)             |
| B                                                                       |                     | 2 (13.3)             |
| C                                                                       |                     | 0 (0)                |
| Not available                                                           | 15 (100)            | 11 (73.33)           |
| <b>METAVIR score, n (%)</b>                                             |                     |                      |
| F0                                                                      | 1 (6.6)             | 1 (12.5)             |
| F1                                                                      | 0 (0)               | 0 (0)                |
| F2                                                                      | 4 (26.6)            | 3 (20)               |
| F3                                                                      | 0 (0)               | 1 (6.6)              |
| F4                                                                      | 3 (20)              | 4 (26.6)             |
| No                                                                      | 0 (0)               | 2 (13.3)             |
| Not available                                                           | 7 (46.6)            | 4 (26.6)             |
| <b>Laboratory [normal values]<br/>(units), mean <math>\pm</math> SD</b> |                     |                      |
| Albumin [3.5 - 5.2] (g/dL)                                              | 4 $\pm$ 0.3         | 3.78 $\pm$ 0.54      |
| <b>Laboratory [normal values]<br/>(units), median [Q1; Q3]</b>          |                     |                      |
| AFP [0.9-7.0] (ng/mL)                                                   | 2.15 [1.72; 4.67]   | 2.5 [1.47; 5.99]     |
| CA 19-9 [2-34] (U/mL)                                                   | 4.5 [2.75; 6]       | 7.52 [4.5; 25]       |
| CEA [0.3-4.7] (ng/mL)                                                   | -                   | 2.71 [1.06; 7.63]    |
| AST [10-50] (U/L)                                                       | 31 [23.5; 66.5]     | 31 [20.5; 64.5]      |
| ALT [10-50] (U/L)                                                       | 43 [22.5; 59]       | 42 [22.5; 53]        |
| $\gamma$ GT [8-61] (U/L)                                                | 112 [51; 322]       | 175 [70; 367.5]      |
| AP [40-129] (U/L)                                                       | 514 [392.5; 853.5]  | 345 [270; 738.5]     |
| TB [0.1-1.2] (mg/dL)                                                    | 0.7 [0.6; 1.1]      | 0.6 [0.4; 1.02]      |
| PT [11.8-14.9] (sec)                                                    | 16.3 [15.15; 19.65] | 15.15 [14.67; 18.77] |
| INR [0.8-1.2]                                                           | 1.04 [0.96; 1.18]   | 1.02 [0.96; 1.44]    |
| Glucose [82-115] (mg/dL)                                                | 89.5 [82; 101.75]   | 85.85 [82; 96.5]     |
| Triglycerides [50-165] (mg/dL)                                          | 112 [90.5; 176]     | 92 [64; 175]         |
| CRP [0-0.5] (mg/dL)                                                     | 0.41 [0.39; 0.73]   | 0.6 [0.41; 1.14]     |
| Cholesterol [110-220] (mg/dL)                                           | 204 [188.5; 255]    | 227 [168; 255]       |

Supplementary Table S1. Baseline characteristics of the study participants

|                                                                         | Discovery cohort<br>CIR (n=15) | Validation cohort |
|-------------------------------------------------------------------------|--------------------------------|-------------------|
| Age (mean $\pm$ SD)                                                     | 64.1 $\pm$ 9.1                 |                   |
| Age [range]                                                             | 51-78                          |                   |
| <b>Gender, n (%)</b>                                                    |                                |                   |
| Males                                                                   | 8 (53.33)                      |                   |
| Females                                                                 | 7 (46.67)                      |                   |
| <b>Environment, n (%)</b>                                               |                                |                   |
| Urban                                                                   | 10 (66.6)                      |                   |
| Rural                                                                   | 5 (33.3)                       |                   |
| <b>Underlying disease, n (%)</b>                                        |                                |                   |
| Hepatitis B virus                                                       | 0 (0)                          |                   |
| Hepatitis C virus                                                       | 1 (6.6)                        |                   |
| Alcohol abuse                                                           | 9 (60)                         |                   |
| Diabetes                                                                | 5 (33.3)                       |                   |
| <b>Child-Pugh score, n (%)</b>                                          |                                |                   |
| A                                                                       | 8 (53.3)                       |                   |
| B                                                                       | 2 (13.3)                       |                   |
| C                                                                       | 2 (13.3)                       |                   |
| Not available                                                           | 3 (20)                         |                   |
| <b>Laboratory [normal values]<br/>(units), mean <math>\pm</math> SD</b> |                                |                   |
| Albumin [3.5 - 5.2] (g/dL)                                              | 3.7 $\pm$ 0.3                  |                   |
| <b>Laboratory [normal values]<br/>(units), median [Q1; Q3]</b>          |                                |                   |
| AFP [0.9-7.0] (ng/mL)                                                   | 2.9 [2.9; 3.3]                 |                   |
| CA 19-9 [2-34] (U/mL)                                                   | 19.25 [10.13; 28.37]           |                   |
| CEA [0.3-4.7] (ng/mL)                                                   | 4.29 [3.79; 4.79]              |                   |
| AST [10-50] (U/L)                                                       | 36.5 [30.5; 40]                |                   |
| ALT [10-50] (U/L)                                                       | 23.5 [19.25; 32.25]            |                   |
| $\gamma$ GT [8-61] (U/L)                                                | 74 [25; 115]                   |                   |
| AP [40-129] (U/L)                                                       | 195 [156; 220.5]               |                   |
| TB [0.1-1.2] (mg/dL)                                                    | 0.98 [0.71; 1.65]              |                   |
| PT [11.8-14.9] (sec)                                                    | 16.6 [15; 18.1]                |                   |
| INR [0.8-1.2]                                                           | 1.24 [1.12; 1.36]              |                   |
| Glucose [82-115] (mg/dL)                                                | 119.5 $\pm$ 43.5               |                   |
| Triglycerides [50-165] (mg/dL)                                          | 105 [66; 109]                  |                   |
| CRP [0-0.5] (mg/dL)                                                     | 1.02 [0.37; 2.18]              |                   |
| Cholesterol [110-220] (mg/dL)                                           | 168 [151; 181]                 |                   |

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|                                                                         | Discovery cohort  | Validation cohort |
|-------------------------------------------------------------------------|-------------------|-------------------|
|                                                                         | HC (n=15)         |                   |
| Age (mean $\pm$ SD)                                                     | 32.1 $\pm$ 8.9    | 35.4 $\pm$ 10.46  |
| Age [range]                                                             | 23-55             | 23-54             |
| <b>Gender, n (%)</b>                                                    |                   |                   |
| Males                                                                   | 9 (60)            | 6 (40)            |
| Females                                                                 | 6 (40)            | 9 (60)            |
| <b>Laboratory [normal values]<br/>(units), mean <math>\pm</math> SD</b> |                   |                   |
| Albumin [3.5 - 5.2] (g/dL)                                              | 3.8 $\pm$ 0.2     | 4.44 $\pm$ 0.39   |
| <b>Laboratory [normal values]<br/>(units), median [Q1; Q3]</b>          |                   |                   |
| CRP [0-0.5] (mg/dL)                                                     | 0.48 [0.35; 0.99] | 0.18 [0.11; 0.27] |

iCCA = intrahepatic cholangiocarcinoma; HCC = hepatocellular carcinoma; PSC = primary sclerosing cholangitis; CIR = liver cirrhosis; HC = healthy controls; n= number; F0-F4 = fibrosis degree; AFP = alpha-fetoprotein; CA 19-9 = carbohydrate antigen 19-9; CEA = carcinoembryonic antigen; AST = aspartate aminotransferase; ALT = alanine aminotransferase;  $\gamma$ GT = gamma-glutamyl transferase; AP = alkaline phosphatase; TB = total bilirubin; PT = prothrombin time; INR = international normalized ratio; CRP = C-reactive protein; normal values are in square brackets; SD = standard deviation, Q = quartile