# **Supplementary material**





CI: 0.880-0.961

1.0

0.2 0.4 0.6 0.8 1-Specificity (FPR)

0.0

**S**3



# S4

| cancer (OS) | total (N) | HR(95%CI)              |                       | P value  |
|-------------|-----------|------------------------|-----------------------|----------|
| ACC         | 12.404    | 4.392 (1.798 - 10.725) |                       | 0.0012   |
| BLCA        | 1.0697    | 1.173(0.869 - 1.582)   | NH<br>NH              | 0.2979   |
| BRCA        | 0.75869   | 0.846(0.577 - 1.240)   | nda -                 | 0.3909   |
| CESC        | 2.9107    | 1.51(0.946 - 2.411)    | <mark>¦</mark> ∙-1    | 0.084    |
| CHOL        | 0.53435   | 1.437(0.551 - 3.747)   | H <mark>e−−−1</mark>  | 0.458    |
| COAD        | 1.0103    | 1.246(0.806 - 1.925)   | NG-H                  | 0.3225   |
| DLBC        | 1.2381    | 2.433(0.535 - 11.057)  | ,<br>                 | 0.2497   |
| ESCA        | 2.2176    | 1.472(0.877 - 2.471)   | ¦∙i                   | 0.1436   |
| GBM         | 2.1403    | 1.318(0.916 - 1.897)   | I<br>₩₽-I             | 0.1371   |
| HNSC        | 1.5717    | 1.207(0.903 - 1.615)   |                       | 0.2043   |
| KIRC        | 8.635     | 1.652(1.195 - 2.283)   | h <del>o</del> i      | 0.0024   |
| KIRP        | 6.3226    | 2.191(1.205 - 3.983)   | -∙•i                  | 0.0101   |
| LAML        | 2.5159    | 0.688(0.438 - 1.080)   | <b>N</b>              | 0.1042   |
| LGG         | 14.992    | 2.017(1.429 - 2.846)   |                       | 6.57e-05 |
| LIHC        | 10.741    | 1.787 (1.259 - 2.535)  | '<br>'⊨●-             | 0.0012   |
| LUAD        | 5.8104    | 1.452 (1.065 - 1.980)  | <b> </b> <del> </del> | 0.0183   |
| LUSC        | 3.3395    | 0.754 (0.560 - 1.014)  |                       | 0.0662   |
| MESO        | 7.8237    | 1.979 (1.235 - 3.173)  |                       | 0.0046   |
| OV          | 5.1307    | 1.371(1.048 - 1.794)   | 1<br>101              | 0.0214   |
| PAAD        | 4.3366    | 0.615(0.382 - 0.989)   |                       | 0.0448   |
| PCPG        | 3.5733    | 4.260 (0.830 - 21.850) | l<br>1 ●              | → 0.0824 |
| PRAD        | 2.3696    | 2.956(0.785 - 11.134)  | ₩ <b></b>             | 0.1092   |
| READ        | 5.0098    | 0.383(0.169 - 0.870)   | 10-11<br>10-1         | 0.0218   |
| SARC        | 5.6895    | 1.780(1.079 - 2.938)   | <b>h</b> ori          | 0.0241   |
| SKCM        | 4.2332    | 1.373(1.007 - 1.872)   | e-                    | 0.0447   |
| STAD        | 2.2186    | 0.778(0.560 - 1.081)   | n,                    | 0.1347   |
| TGCT        | 0.070648  | 1.306(0.183 - 9.331)   |                       | 0.7901   |
| THCA        | 1.6542    | 2.153(0.612 - 7.569)   |                       | 0.232    |
| THYM        | 6.8897    | 0.140(0.027 - 0.721)   | ●H                    | 0.0188   |
| UCEC        | 7.2933    | 1.860(1.160 - 2.982)   | <b>⊢</b> •            | 0.0099   |
| UCS         | 2.2283    | 0.590(0.294 - 1.186)   | len<br>I              | 0.1387   |
| UVM         | 4.0462    | 4.929(0.663 - 36.631)  | µ <b></b>             | → 0.119  |
|             |           |                        | 0.0 2.5 5.0 7.5 10.0  | 12.5     |

| C | F |
|---|---|
| S | J |

|  | total (N) HR(95%CI)            | 5%CI)           | P value  |
|--|--------------------------------|-----------------|----------|
| · · · · ·                                | 12.019 4.583 (1.781 - 11.789)  | 81 - 11         | 0.0016   |
| ie-i                                     | 3.325 1.409(0.968 - 2.049)     | 68 - 2.0        | 0.0731   |
|  | 6.6033 0.479(0.260 - 0.883)    | 50 - 0.8        | 0.0182   |
| <b> </b> ⊷-i                             | 4.1214 1.742(1.025 - 2.961)    | 25 - 2.9        | 0.0401   |
|  | 1.0345 0.531(0.167 - 1.691)    | 67 - 1.0        | 0.2843   |
| н  | 0.4026 0.847(0.505 - 1.420)    | )5 <b>-</b> 1.4 | 0.5291   |
| ÷  | 1.533 3.625(0.502 - 26.172)    | 2 - 26          | → 0.2016 |
| 1  | 4.0695 1.870(1.870)            | 1.870)          | 0.0506   |
| ,<br>Me+i                                | 2.5454 1.375(0.923 - 2.049)    | 23 - 2.0        | 0.1174   |
| <b>10</b> -1                             | 2.4489 1.344(0.934 - 1.935)    | 34 - 1.9        | 0.1114   |
| He-H                                     | 6.8205 1.673 (1.143 - 2.449)   | 43 - 2.         | 0.008    |
| · · · · · · · · · · · · · · · · · · ·    | 4.4022 2.751(0.954 - 7.929)    | 54 - 7.9        | 0.0611   |
| •  | 0 0                            | )               | 0        |
| He-H                                     | 13.26 1.998 (1.392 - 2.869)    | 92 - 2.         | 2e-04    |
|  | 13.226 2.308(1.481 - 3.599)    | 31 - 3.         | 2e-04    |
| }e⊣                                      | 5.7631 1.582 (1.080 - 2.319)   | 80 - 2.         | 0.0186   |
| <mark>¦e</mark> ⊣                        | 1.5012 1.337 (0.832 - 2.147)   | 32 - 2.         | 0.2298   |
|  | 6.84 2.257(1.220 - 4.176)      | 20 - 4.         | 0.0095   |
| ei i                                     | 3.7955 1.341(1.003 - 1.794)    | )3 - 1.         | 0.0479   |
|  | 4.4591 0.607(0.381 - 0.965)    | 31 - 0.9        | 0.035    |
| <b>├</b> •                               | 3.9284 5.257(0.956 - 28.923)   | 6 - 28          | → 0.0564 |
| ⊢ <mark>, ●</mark>                       | 0.91184 2.508 (0.411 - 15.293) | 1 - 15          | → 0.3189 |
| He H | 0.70189 0.618(0.194 - 1.972)   | 94 - 1.9        | 0.4165   |
|  | 3.2878 1.606(0.941 - 2.740)    | 41 - 2.         | 0.0826   |
| ei .                                     | 3.8325 1.378 (0.992 - 1.916)   | 92 - 1.         | 0.0562   |
| ¦<br>₩●──I                               | 2.2198 1.499(0.860 - 2.615)    | 60 - 2.0        | 0.1536   |
| · · ·                                    | 0.73838 2.759(0.250 - 30.450)  | 0 - 30.         | 0.4074   |
| •  | 0 0                            | )               | 0        |
|  | 2.0921 0.198(0.023 - 1.684)    | 23 - 1.0        | 0.1382   |
| <b>↓</b> ● 1                             | 5.098 1.785(1.087 - 2.930)     | 37 - 2.9        | 0.022    |
| H<br>H                                   | 1.6878 0.619(0.298 - 1.283)    | 98 - 1.2        | 0.1967   |
|  | 3.4957 2.453(0.997 - 6.033)    | 97 - 6.0        | 0.0507   |

# Figure S6 S6

| cancer (PFI) | total (N) | HR(95%CI)              |                   | P value  |
|--------------|-----------|------------------------|-------------------|----------|
| ACC          | 19.087    | 4.701( 2.189 - 10.093) | ↓ <b>→ → → →</b>  | 7.19e-05 |
| BLCA         | 6.4558    | 1.488( 1.089 - 2.033)  | 10-1              | 0.0127   |
| BRCA         | 3.687     | 0.71(0.503 - 1.000)    | •                 | 0.0499   |
| CESC         | 9.5098    | 2.087(1.314 - 3.314 )  | ,<br>He-H         | 0.0018   |
| CHOL         | 0.32361   | 0.737(0.265 - 2.052)   | ₩                 | 0.5597   |
| COAD         | 0.91526   | 1.201(0.822 - 1.756)   | μ.                | 0.3444   |
| DLBC         | 3.87      | 3.831(1.019 - 14.397)  |                   | 0.0468   |
| ESCA         | 1.7119    | 1.355(0.855 - 2.149)   | <b>10</b> -1      | 0.1962   |
| GBM          | 1.3983    | 0.814(0.578 - 1.146)   |                   | 0.2391   |
| HNSC         | 4.4371    | 1.384(1.028 - 1.864)   | lei               | 0.032    |
| KICH         | 8.9719    | 6.584(1.738 - 24.939)  | ¦                 | → 0.0055 |
| KIRC         | 1.2575    | 1.208 (0.871 - 1.677)  | <b>h</b>          | 0.2572   |
| KIRP         | 3.946     | 1.717(1.014 - 2.909)   | <b>HHH</b>        | 0.0444   |
| LAML         | 0         | 0                      | •                 | 0        |
| LGG          | 5.2554    | 1.403 (1.056 - 1.865)  | lei               | 0.0196   |
| LIHC         | 22.557    | 2.033(1.518 - 2.721)   | нен               | 1.9e-06  |
| LUAD         | 5.4879    | 1.392 (1.050 - 1.846)  | ы                 | 0.0214   |
| LUSC         | 2.3329    | 1.289 (0.930 - 1.786)  | <b>P</b>          | 0.1272   |
| MESO         | 3.1304    | 1.650(0.961 - 2.833)   | ¦<br>₩            | 0.0693   |
| OV           | 1.8388    | 1.192 (0.927 - 1.533)  |                   | 0.1708   |
| PAAD         | 8.6155    | 0.554(0.374 - 0.822)   |                   | 0.0033   |
| PCPG         | 4.9044    | 2.627(1.135 - 6.081)   | <b>┝</b> ━━━━┥    | 0.0241   |
| PRAD         | 8.0505    | 1.810(1.207 - 2.716)   | he-t              | 0.0041   |
| READ         | 2.2422    | 1.872(0.779 - 4.502)   | ¦ <b>↓</b> → → ↓  | 0.1612   |
| SARC         | 1.2555    | 1.235(0.859 - 1.775)   | <b>P</b> H        | 0.2551   |
| SKCM         | 4.8027    | 1.319(1.024 - 1.699)   |                   | 0.0318   |
| STAD         | 1.7935    | 1.317 (0.872 - 1.988)  | Mo-1              | 0.1904   |
| TGCT         | 1.299     | 1.439 (0.768 - 2.696)  | H <del>o</del> -1 | 0.2561   |
| THCA         | 2.741     | 0.633(0.371 - 1.082)   | •                 | 0.0945   |
| THYM         | 2.4697    | 0.492(0.205 - 1.179)   | ■ <mark>/</mark>  | 0.1116   |
| UCEC         | 2.9634    | 1.369(0.962 - 1.949)   |                   | 0.0812   |
| UCS          | 1.0479    | 0.709(0.367 - 1.371)   | юн                | 0.3066   |
| UVM          | 8.8997    | 5.566(1.318 - 23.498)  | ·<br>·            | → 0.0195 |
|              |           |                        | 0 5 10            | 15       |













































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#### **Figure legends**

#### FigureS1.

DBF4B expression from HPA database. (A) DBF4B expression in normal cell lines from the HPA database. (B) Plots of single-cell RNA-sequencing data from the FUCCI U-2 cell line showing the correlation between DBF4B RNA expression and cell cycle progression.

#### FigureS2.

Immunofluorescence staining of the subcellular localization of DBF4B in HPA database.

#### FigureS3.

Diagnostic value of DBF4B in pan-cancer using AUC of ROC curves response based on TCGA data

#### FigureS4.

Forest plot of OS associations in pan cancer.

#### FigureS5.

Forest plot of DSS associations in pan cancer.

#### FigureS6.

Forest plot of PFI associations in pan cancer.

#### FigureS7.

Correlations between DBF4B expression and molecular subtypes across TCGA cancers. CIN, chromosomal instability; GS, genomically stable; POLE, Polymerase ɛ; EBV, Epstein-Barr virus.C1: wound healing, C2: IFN-gamma dominant, C3: inflammatory, C4: lymphocyte depleted, C5: immunologically quiet, and C6: TGF-b dominant.

#### FigureS8.

Correlations between DBF4B expression and immune subtypes across TCGA cancers. CIN, chromosomal instability; GS, genomically stable; POLE, Polymerase ε; EBV, Epstein-Barr virus.C1: wound healing, C2: IFN-gamma dominant, C3: inflammatory, C4: lymphocyte depleted, C5: immunologically quiet, and C6: TGF-b dominant. **Figure S9.** 

Scatter plot of correlation between DBF4B expression and immune score.

#### FigureS10.

Scatterplot of correlation between DBF4B expression and stromal score.

FigureS11.

Scatterplot of correlation between DBF4B expression and estimated score.

#### FigureS12.

The correlation of DBF4B expression with TMB.

## FigureS13.

The correlation of DBF4B expression with MSI.

### FigureS14.

The correlation of DBF4B expression with DNAss.

## FigureS15.

The correlation between DBF4B expression and m1A, m5C, m6A regulatory genes. \*: p < 0.05.

## FigureS16.

Chromosomal distribution of the methylation probes associated with DBF4B.

## FigureS17.

Association of GSCALite-based expression of DBF4B and related genes with drug sensitivity.

## FigureS18.

Associations between DBF4B expression and the OS in different clinical subgroups of LIHC. (A) Age > 60; (B) BMI  $\leq 25$ ; (C) Race: Asian; (D) Gender: male; (E) Residual tumor: R0; (F) Histological type: Hepatocellular carcinoma; (G) Pathological stage: Stage III; (H) Tumor state: With tumor; (I) Pathological T stage: T3.

## FigureS19.

Associations between DBF4B expression and the DSS in different clinical subgroups of LIHC. (A) Age > 60; (B) BMI  $\leq$  25; (C) Race: Asian; (D) Gender: male; (E) Residual tumor: R0; (F) Histological type: Hepatocellular carcinoma; (G) Pathological stage: Stage III; (H) Tumor state: With tumor; (I) Pathological T stage: T3.

## FigureS20.

Associations between DBF4B expression and the PFI in different clinical subgroups of LIHC. (A) Age > 60; (B) BMI  $\leq$  25; (C) Race: Asian; (D) Gender: male; (E) Residual tumor: R0; (F) Histological type: Hepatocellular carcinoma; (G) Tumor state : With tumor; (H) Vascular invasion: Yes; (I) Albumin(g/dl): >= 3.5.

## FigureS21.

Analysis of differentially expressed genes and functional enrichment of DBF4B in LIHC. (A) Volcano plot of DEGs, with up-regulated genes in red and down-regulated genes in blue. (B) GO annotates five classes of pathways positively associated with high levels of DBF4B expression. (C) GO annotates five classes of pathways negatively associated with high levels of DBF4B expression. (D) KEGG analysis of five pathways positively associated with DBF4B expression. (E) KEGG analysis of five pathways negatively associated with DBF4B expression.

## Table S1

# The clinical parameters of 30 patients

| Pati<br>ents | Gen<br>der | A<br>g<br>e | Cance<br>r<br>type | CN<br>LC | BC<br>LC | Child<br>pugh | AFP(n<br>g/m1) | Vasc<br>ular<br>Tumo<br>r<br>thro<br>mbus | M<br>V<br>I | Smoking<br>history | Alcohol<br>history | H<br>B<br>V | hypert<br>ension | diab<br>etes | Hea<br>rt<br>dis<br>eas<br>e | His<br>tor<br>y<br>of<br>sur<br>ger<br>y | Othe<br>r<br>Dise<br>ases |
|--------------|------------|-------------|--------------------|----------|----------|---------------|----------------|---|-------------|--------------------|--------------------|-------------|------------------|--------------|------------------------------|--|---------------------------|
| 1            | mal<br>e   | 5<br>4      | HCC                | IA       | A        | А             | 68.37          | 0   | M<br>O      | 1                  | 1                  | 1           | 0                | 0            | 0                            | 0  | 0                         |
| 2            | fem<br>ale | 6<br>6      | HCC                | IB       | В        | А             | 59.37          | 0   | M<br>O      | 0                  | 0                  | 1           | 0                | 0            | 0                            | 0  | 0                         |
| 3            | mal<br>e   | 6<br>9      | HCC                | IB       | В        | А             | 195.3<br>5     | 1   | M<br>O      | 1                  | 0                  | 1           | 0                | 0            | 0                            | 0  | 0                         |
| 4            | mal<br>e   | 7<br>2      | HCC                | IB       | В        | А             | 67.89          | 0   | M<br>O      | 1                  | 1                  | 1           | 0                | 0            | 0                            | 0  | 0                         |
| 5            | mal<br>e   | 5<br>1      | HCC                | IA       | А        | А             | 197.5<br>9     | 1   | M<br>O      | 1                  | 1                  | 1           | 0                | 0            | 0                            | 1  | 0                         |
| 6            | fem<br>ale | 7<br>0      | HCC                | IA       | А        | А             | 65.38          | 0   | M<br>O      | 0                  | 0                  | 1           | 0                | 0            | 0                            | 0  | 0                         |
| 7            | mal<br>e   | 4<br>4      | HCC                | IB       | В        | А             | 59.49          | 0   | M<br>1      | 1                  | 1                  | 1           | 0                | 0            | 0                            | 1  | 0                         |
| 8            | mal<br>e   | 4<br>8      | HCC                | IA       | А        | А             | 65.36          | 1   | M<br>O      | 1                  | 1                  | 1           | 0                | 0            | 0                            | 0  | 0                         |
| 9            | mal<br>e   | 5<br>9      | HCC                | IA       | А        | А             | 59.59          | 0   | M<br>O      | 1                  | 1                  | 1           | 0                | 0            | 0                            | 1  | 0                         |
| 10           | mal<br>e   | 7<br>1      | HCC                | II<br>B  | В        | А             | 65.34          | 0   | M<br>O      | 0                  | 0                  | 1           | 1                | 1            | 0                            | 0  | 1                         |
| 11           | mal<br>e   | 3<br>9      | HCC                | IA       | А        | А             | 46.56          | 0   | M<br>O      | 0                  | 0                  | 1           | 0                | 0            | 0                            | 0  | 0                         |

| 12 | mal<br>e   | 4<br>3 | HCC | IA      | А | А | 2.63        | 0 | M<br>1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
|----|------------|--------|-----|---------|---|---|-------------|---|--------|---|---|---|---|---|---|---|---|
| 13 | mal<br>e   | 5<br>7 | HCC | IA      | A | А | 78.36       | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 14 | fem<br>ale | 7<br>2 | HCC | II<br>B | В | А | 123. 3<br>6 | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 15 | mal<br>e   | 3<br>5 | HCC | IA      | A | А | 65.39       | 0 | M<br>O | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 16 | fem<br>ale | 4<br>8 | HCC | II<br>B | В | А | 477.3<br>2  | 0 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 17 | mal<br>e   | 3<br>4 | HCC | IA      | A | А | 68.36       | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 18 | mal<br>e   | 5<br>7 | HCC | IA      | A | А | 135.3<br>6  | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 19 | fem<br>ale | 7<br>2 | HCC | IA      | A | А | 89.57       | 0 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 20 | mal<br>e   | 5<br>3 | HCC | IA      | A | А | 75.5        | 1 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 21 | fem<br>ale | 4<br>4 | HCC | IA      | A | А | 2.89        | 0 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 22 | mal<br>e   | 4<br>5 | HCC | II<br>B | В | А | 89.45       | 0 | M<br>1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 23 | mal<br>e   | 6<br>0 | HCC | IA      | A | А | 45.68       | 0 | M<br>O | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| 24 | fem<br>ale | 5<br>9 | HCC | IA      | A | А | 84.54       | 0 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 25 | fem<br>ale | 6<br>7 | HCC | II<br>B | В | А | 356.4<br>5  | 0 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 36 | mal<br>e   | 4<br>4 | HCC | IA      | A | А | 56.54       | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 37 | fem<br>ale | 6<br>9 | HCC | IA      | A | А | 86.65       | 0 | M<br>1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 38 | fem<br>ale | 4<br>1 | HCC | IA      | A | А | 68.98       | 0 | M<br>O | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 39 | mal<br>e   | 5<br>6 | HCC | II<br>B | В | А | 59.67       | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 30 | mal<br>e   | 6<br>7 | HCC | IA      | А | А | 167.6<br>5  | 0 | M<br>O | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |