Supporting Information

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S100A9 Derived from Chemoembolization-Induced Hypoxia Governs Mitochondrial Function in Hepatocellular Carcinoma Progression

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S100A9 derived from chemoembolization-induced hypoxia governs mitochondrial function in hepatocellular carcinoma progression

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Catalogue

1. Supplementary figures 1-6 and tables 1-2
2. CRISPR Knockout Pooled Library.xls
3. Ischemia VS ConGene_differential_expression.xlsx
4. TACE_ER VS LR_Gene_differential_expression.xlsx
5. P-PLCPRF5-CTR-vs-P-PLCPRF5-sh1.genes. annot
6. Antibody, prime and chemicals reagents
1. Supplementary figures 1-6 and tables 1-2

Supplementary figure 1

A

Statistics of Pathway Enrichment

B

Supplementary figure 1

C

Supplementary figure 1

D

Supplementary figure 1

E

Supplementary figure 1

F

Supplementary figure 1

G

Supplementary figure 1

H

Supplementary figure 1

I

Supplementary figure 1

J

Supplementary figure 1

K

Supplementary figure 1

L

Supplementary figure 1

M

Supplementary figure 1

N

Supplementary figure 1

O

Supplementary figure 1

P

Supplementary figure 1

Q

Supplementary figure 1

R

Supplementary figure 1
Supplementary figure 1 S100A9 is a key driver of post-TACE HCC progression.
A) KEGG enrichment analysis of DEGs in HCC tissues in patients between the early recurrence and late recurrence groups. B) Heatmap showing the DEGs between early recurrence and late recurrence (showing only the 10 most upregulated genes in each group) (tumor recurrence within two years after surgical resection). C) Heatmap showing the DEGs between the ischemia group and the control group (showing only the 10 most upregulated genes in each group). D) Heatmap showing the DEGs between day 0 and day 14 from the CRISPR/Cas9 library screen in PLC/PRF/5 cells (showing only the 10 most upregulated genes in each group). E) Flow cytometry showing the proportion of EPCAM+/S100A9+ and CD45+/S100A9+ cells among total S100A9+ cells in the HCC tissues of patients who received only surgery (top) or patients who received TACE followed by surgery (bottom). F) TACE upregulated the proportion of EPCAM+/S100A9+ cells and downregulated the proportion of CD45+/S100A9+ cells among the total S100A9+ cells in the HCC tissues of patients who received TACE followed by surgery compared to patients who received surgery only (n=6 each group). G-J) Multiple immunofluorescence staining showing DAPI (gray), S100A9 (red), EPCAM (green, Fig G), CD45 (yellow, Fig H), CD68 (bright blue, Fig I) and CD3 (blue, Fig J) expression, coexpression (double-positive cells) and quantification of each kind of cell proportion in the tumor regions in patients treated with or without TACE (three random areas for five different samples). K) RNA expression of S100A9 in orthotopic liver xenograft tumors after different treatments in vivo. Epi (epirubicin), Cis (cisplatinum), Isc (ischemia). L) RNA expression of S100A9 in MHCC-97H cells after different treatments in vitro. H (hypoxia) (1% oxygen 24 hours). M) Hypoxia stabilized HIF1A and induced S100A9 expression in Hep-3B cells. N) Silencing HIF1A suppressed the expression of S100A9 induced by hypoxia in MHCC-97H cells. O) Schematic view of the luciferase reporter constructs containing various lengths of the 5′-flanking regions of the S100A9 promoter. Detailed characterization of the S100A9 promoter by 5′-deletion and site-specific deletion analyses was performed. P) S100A9 promoter luciferase activity in PLC/PRF/5 cells or 293T cells cotransfected with the vector or the indicated luciferase reporter and pcdna3.1-HIF1A constructs for 48 h. Q) Representation of S100A9 promoter regions and the indicated primers for ChIP assay. R) ChIP assay for HIF1A occupancy at the S100A9 promoter in PLC/PRF/5 cells or Huh7 cells. Precipitated DNA was purified and subjected to semiquantitative PCR. Data in (F)-(L) and (P) is presented as mean ± SEM, * P<0.05, ** P < 0.01, *** P < 0.001, by two-tailed unpaired Student t-test.
Supplementary figure 2

A - B: Graphs showing expression levels of PLCPRF5 and HUH7 in different conditions.

D - E: Images and graphs illustrating the effects of various treatments on cell lines.

F - G: Comparative analysis of PLCPRF5 and HUH7 expression in different experimental groups.

H - I: Morphological analysis and quantification of cell populations.

J - K: Western blots and immunoblotting results for E-cadherin and N-cadherin expression.

L - M: Gene expression analysis and correlation of marker genes with fibrosis.

N - O: Histological images of different experimental conditions.

P - Q: Additional histological analysis and comparison of fibrosis levels.

R - S: Additional images and data for fibrosis evaluation.

T: Statistical graph showing the number of fibrosis foci in different conditions.

Overall, the figures illustrate the effects of various treatments on cell line expression and fibrosis levels, demonstrating the importance of PLCPRF5 and HUH7 in these processes.
Supplementary figure 2 S100A9 promotes the growth and metastasis of HCC cells in vitro and in vivo.

A) Real-time PCR (left) and Western blotting assays (right) showing the expression of S100A9 in hepatoma cell lines (n=3). B-C) Real-time PCR and Western blot assays showing the efficiency of S100A9 knockout/overexpression in PLC/PRF/5 (B) and MHCC-97H (C) cells (n=3). D) Tas inhibited the expression of S100A9 in HCC cells. PLC/PRF/5 cells were treated with Tas at the indicated concentration for 24 h before harvest. MHCC-97H-OE and Huh7 cells were treated with 100 µM Tas for 24 h before harvesting. E) Tas inhibited the proliferation of MHCC-97H-OE (left) and Huh7 (right) cells, as indicated by CCK-8 assays (n=3). F-G) Knockout of S100A9 suppressed and overexpression of S100A9 enhanced the migration of HCC cells, as indicated by wound-healing assays. H-J) Knockout of S100A9 or 100 µM Tas inhibited the cell migration of HCC cells, as indicated by Transwell assays (n=3). K) Western blot analysis showing the expression of S100A9 and EMT markers in Huh7/S100A9-sg cells. L) Overexpression of S100A9 enhanced the metastatic ability of tumor cells, even after neutralizing CD11b-positive cells in C57 mice, which were measured by in vivo bioluminescent imaging (n=5 each group). M) Quantification of photon flux as the mean ± SEM. N) Knockout of S100A9 significantly suppressed the lung metastatic capacity of PLC/PRF/5. O) Representative E-cadherin and fibronectin staining in lung metastases of PLC/PRF/5-sg or control cells. P) Tas suppressed the lung metastatic capacity of PLC/PRF/5. Q) Representative E-cadherin and fibronectin staining in lung metastases of Tas-treated groups and control groups. R) Overexpression of S100A9 promoted the lung metastatic capacity of MHCC-97H cells. S) Representative E-cadherin and fibronectin staining in lung metastases of MHCC-97H-OE or control cells. T) Overexpression of S100A9 promoted the lung metastatic capacity of HCC cells and decreased E-cadherin expression and increased fibronectin expression in lung metastases (n=8 each group). Data in (A)-(E), (H)-(J), (M) and (T) are presented as mean ± SEM, * P < 0.05, ** P < 0.01, ***P<0.001, by two-tailed unpaired Student t-test.
Supplementary figure 3 S100A9 modulates cellular mitochondrial function and promotes ROS production.

A.) S100A9 knockout reduced intracellular ROS production in Huh7 cells, as indicated by DCFH-DA fluorescence assay. B-D) Tas reduced intracellular ROS production in PLC/PRF/5 (B), Huh7 (C) and MHCC-97H (D) cells. E-F) Mdivil-1 (10 μM) reduced intracellular ROS production in PLC/PRF/5 (E) and MHCC-97H (F) cells. G) Effects of S100A9 on oxidative phosphorylation (as determined by Seahorse XF analyzers) in PLC/PRF/5 (top) and MHCC-97H cells (bottom). The oxygen consumption rate (OCR) over time were shown. H) Effects of S100A9 on glycolytic rates (as determined by Seahorse XF analyzers) in PLC/PRF/5 (top) and MHCC-97H cells (bottom). The extracellular acidification rate (ECAR) (left) over time and the calculated glycolytic rates(right) were shown. I) S100A9 knockout reduced whereas S100A9 overexpression increased ATP production in HCC cells. J-K) Knockout of S100A9 or overexpression of S100A9 did not consistently change the RNA levels of the NOX family, as indicated by real-time PCR. L) Mito-tempo (20 μM) reduced intramitochondrial ROS production in MHCC-97H/S100A9-OE cells. M) Decreased intramitochondrial ROS with 20 μM Mito-tempo inhibited the proliferation ability of MHCC-97H/S100A9-OE cells. N) Decreased intramitochondrial ROS with 20 μM Mito-tempo inhibited the migration ability of MHCC-97H/S100A9-OE cells. Data in (A)-(N) are presented as mean ± SEM, n=3, * P < 0.05, ** P < 0.01, ***P<0.001, by two-tailed unpaired Student t-test.
Supplementary figure 4 S100A9 recruits USP10 and PGAM5 to maintain PGAM5 stability.

A) Knockdown of PGAM5 inhibited mitochondrial fission in PLC/PRF/5 cells. Scale bars, 20 μm. The proportion of HCC cells (n =100 cells for each sample, repeated three times) with tubulated, intermediate, and fragmented mitochondria was quantified. B) Knockdown of PGAM5 inhibited intracellular ROS production in PLC/PRF/5 cells, as indicated by DCFH-DA fluorescence assay. C) Knockdown of PGAM5 inhibited intramitochondrial ROS production in PLC/PRF/5 cells, as indicated by MitoSOX fluorescence assay. D) Knockdown of PGAM5 inhibited the proliferation of PLC/PRF/5 cells, as indicated by EdU assay. E) Knockdown of PGAM5 inhibited the migration of PLC/PRF/5 cells, as indicated by Transwell assays. F) Western blot for PGAM5 and PGAM5 target genes and EMT markers in HCC cells. G) Real-time PCR showing the knockdown efficiency of PGAM5 in PLC/PRF/5 cells. H) Co-IP showing the interaction of S100A9 and PGAM5 in Huh7 cells. I) Representative images of immunofluorescence colocalization of S100A9 (red) and PGAM5 (green) in Huh7 cells. J) Real-time PCR showed that S100A9 did not change the RNA level of PGAM5 in HCC cells. K) Knockout of S100A9 decreased the protein level of PGAM5 in Huh7 cells. L) Western blot showing the expression of S100A9 and PGAM5 target genes in PLC/PRF/5 and MHCC-97H cells. S100A9 knockout increased while S100A9 overexpression decreased dephosphorylation at Ser637 of DRP. M) Real-time PCR showing the knockdown efficiency of USP10 in HCC cells. Data in (A)–(E), (G), (J), (M) are presented as mean ± SEM, n = 3. n.s. none sense. ** P < 0.01, *** P < 0.001 by two-tailed unpaired Student t-test.
Supplementary figure 5

A

PLC/PRF/5

NC
S100A9 sg
S100A9 sg + PGAM5

PGAM5
S100A9
Tubulin

B

MHCC-97H

NC
S100A9 sg
S100A9 sg + PGAM5

PGAM5
S100A9
Tubulin

C

Huh7

NC
S100A9 sg
S100A9 sg + PGAM5

PGAM5
S100A9
Tubulin

D

Huh7

NC
S100A9 sg
S100A9 sg + PGAM5

DNA
EDU
Merge

E

Huh7

NC
S100A9 sg
S100A9 sg + PGAM5

NC
S100A9 sg
S100A9 sg + PGAM5

Edu positive cells (%)

F

Huh7

NC
S100A9 sg
S100A9 sg + PGAM5

Counts

G

Huh7

NC
S100A9 sg
S100A9 sg + PGAM5

Counts

ROS

Mitosux
Supplementary figure 5 S100A9 affects HCC growth and metastasis through a PGAM5-dependent pathway.

A) Western blot analysis showing the PGAM5 overexpression efficiency in PLC/PRF/5 cells. B) Western blot analysis showing the PGAM5 knockdown efficiency in MHCC-97H cells. C) Western blot analysis showing the PGAM5 overexpression efficiency in Huh7 cells. D) Ectopic expression of PGAM5 enhanced the growth of S100A9-sg Huh7 cells, as indicated by EdU assay. E) Ectopic expression of PGAM5 enhanced the migration of S100A9-sg Huh7 cells, as indicated by the Transwell assay. F) Ectopic expression of PGAM5 enhanced the intracellular ROS level of Huh7-S100A9-sg cells, as indicated by DCFH-DA fluorescence assay. G) Ectopic expression of PGAM5 enhanced the intramitochondrial ROS level of Huh7-S100A9-sg cells, as indicated by the MitoSOX fluorescence assay. Data in (E)–(G) are presented as mean ± SEM, n = 3. *** P < 0.001 by two-tailed unpaired Student t-test.
Supplementary figure 6 Clinical significance of S100A9 inhibition in HCC.
A) Stacked histogram for AFP levels in the S100A9-High group and S100A9-Low group in the SYSUCC cohort. B) Correlation between S100A9 expression and pathological stage in the SYSUCC cohort (n=172) (grouping by median S100A9 expression based on IHC score). C) Correlation between S100A9 expression and tumor size in the SYSUCC cohort (n=172) (grouping by median S100A9 expression based on IHC score). D-F) Correlation between S100A9 expression and T stage, tumor stage and tumor grade in the TCGA-LIHC cohort. Data in (D)–(F) are presented as mean ± SEM. n=352. Significant differences were determined by one-way ANOVA. G) Kaplan–Meier curve analysis of OS in HCC patients by the expression of S100A9 in the TCGA-LIHC cohort (n=352) (grouping by median S100A9 expression). H) Representative HE staining of lung metastases (left) and quantification of the tumor number of lung metastases (right) of PLC/PRF/5 cells treated with different drugs as indicated. Data in (H) are presented as mean ± SEM. n=5. n.s. none sense, * P < 0.05, *** P<0.001 by one-way ANOVA. I) Western blot analysis showing the inhibition efficiency of S100A9 by RNAi in HCC-PDXs. J) Western blot analysis showing the inhibition efficiency of Tas in HCC-PDXs. K) Tumor weight of HCC-PDXs treated with in vivo-optimized siRNA. L) Tumor weight of HCC-PDXs treated with Tas. Data in (K) and (L) are presented as mean ± SEM, n = 6. * P < 0.05 by two-tailed unpaired Student t-test.

Supplementary Table 1 Correlation between S100A9 and clinicopathological parameters

| Characteristics          | S100A9 low (n = 88) | S100A9 High (n = 84) | P value |
|--------------------------|---------------------|----------------------|---------|
| Age                      |                     |                      |         |
| ≤50                      | 43(48.8%)           | 41(48.8%)            | 0.994   |
| >50                      | 45(51.2%)           | 43(51.2%)            |         |
| Sex                      |                     |                      | 0.438   |
| Male                     | 77(87.5%)           | 70(83.3%)            |         |
| Female                   | 11(12.5%)           | 14(16.7%)            |         |
| HBV                      |                     |                      | 0.928   |
| Yes                      | 81(92.0%)           | 77(91.7%)            |         |
| No                       | 7(8.0%)             | 7(8.3%)              |         |
| AFP                      |                     |                      | 0.001   |
| ≥400 ng/ml               | 23(26.1%)           | 42(50%)              |         |
| <400 ng/ml               | 65(73.9%)           | 42(50%)              |         |
| Tumour size              |                     |                      | 0.042   |
| <10cm                    | 75(85.2%)           | 61(72.6%)            |         |
| ≥10cm                    | 13(14.8%)           | 23(27.4%)            |         |
| Complete capsule         |                     |                      | 0.888   |
| Yes                      | 15(17.0%)           | 15(17.9%)            |         |
| No                       | 73(83.0%)           | 69(82.1%)            |         |
| Microvascular invasion   |                     |                      | 0.864   |
| Yes                      | 23(26.1%)           | 21(25%)              |         |
| No                       | 65(73.9%)           | 63(75%)              |         |
| Margin                   |                     |                      | 0.004   |
|         | Yes          | No          |
|---------|--------------|-------------|
| Clear   | 66 (75.0%)   | 22 (25.0%)  |
| Cirrhosis (yes/no) |  |  |
| Yes     | 55 (65.5%)   | 29 (34.5%)  |
| No      | 66 (75.0%)   | 22 (25.0%)  |
| CA19_9  |              |             |
| ≥35 U/mL| 20 (22.7%)   | 25 (29.8%)  |
| <35 U/mL| 68 (77.3%)   | 59 (70.2%)  |
| CEA     |              |             |
| ≥5 ng/mL| 16 (18.2%)   | 8 (9.5%)    |
| <5 ng/mL| 72 (81.8%)   | 76 (90.5%)  |
| ALB     |              |             |
| >35 g/L | 83 (94.3%)   | 76 (90.5%)  |
| ≤35 g/L | 5 (5.7%)     | 8 (9.5%)    |
| ALT     |              |             |
| >50U/L  | 29 (33.0%)   | 30 (35.7%)  |
| ≤50 U/L | 59 (67.0%)   | 54 (64.3%)  |
| AST     |              |             |
| >40U/L  | 39 (44.3%)   | 38 (45.2%)  |
| ≤40U/L  | 49 (55.7%)   | 46 (54.8%)  |
| TBIL    |              |             |
| >17.1μmol/L | 28 (31.8%) | 17 (20.2%) |
| ≤17.1μmol/L | 60 (68.2%) | 67 (79.8%) |

HBV, Hepatitis B virus; AFP, α-fetoprotein; CA19-9, Carbohydrate antigen 19-9; CEA, Carcinoma Embryonic Antigen; ALB, Albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TBIL, Total bilirubin
### Supplementary Table 2 Univariate and Multivariate Cox analysis of factors associated with overall survival of HCC patients in SYSUCC cohort

| Characteristic                  | Univariate          |          | Multivariate        |          |
|--------------------------------|---------------------|----------|---------------------|----------|
|                                | HR (95% CI)         | P        | HR (95% CI)         | P        |
| Age > 50 (yes/no)              | 0.844(0.498-1.431)  | 0.530    |                     |          |
| Sex (male/female)              | 1.205(0.545-2.662)  | 0.645    |                     |          |
| Tumor size (>10 cm) (yes/no)   | 1.888(0.995-3.582)  | 0.052    |                     |          |
| Margin unclarity (yes/no)      | 1.758(0.884-3.494)  | 0.108    |                     |          |
| Complete capsule (yes/no)      | 1.366(0.663-2.818)  | 0.398    |                     |          |
| Microvascular invasion (yes/no)| 1.818(1.049-3.151)  | 0.033    | 1.845(1.060-3.211)  | 0.03     |
| HBV infection (yes/no)         | 0.619(0.265-1.449)  | 0.269    |                     |          |
| IHC scores of S100A9 (high/low)| 1.956(1.136-3.370)  | 0.016    | 2.057(1.192-3.548)  | 0.01     |
| IHC scores of PGAM5 (high/low) | 1.891(1.081-3.308)  | 0.025    |                     |          |
| AFP > 400 ng/ml (yes/no)       | 1.496(0.882-2.536)  | 0.135    |                     |          |
| CA19_9 > 35 U/mL (yes/no)      |                     |          |                     |          |
| CEA > 5 ng/mL (yes/no)         | 0.671(0.287-1.568)  | 0.357    |                     |          |
| Cirrhosis (yes/no)             | 0.830(0.471-1.464)  | 0.52     |                     |          |
| ALB > 35 g/L (yes/no)          | 0.562(0.238-1.164)  | 0.113    |                     |          |
| ALT > 50 U/L (yes/no)          | 2.340(1.384-3.956)  | 0.002    | 2.445(1.443-4.144)  | 0.001    |
| AST > 40 U/L (yes/no)          | 2.339(1.365-4.008)  | 0.002    |                     |          |
| TBIL > 17.1μmol/L (yes/no)     | 1.300(0.724-2.334)  | 0.379    |                     |          |

HR, Hazard ratio; CI, Confidence interval; HBV, Hepatitis B virus; AFP, α-fetoprotein; CA19-9, Carbohydrate antigen 19-9; CEA, Carcinoma Embryonic Antigen; ALB, Albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TBIL, Total bilirubin

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|-------------------|---------------------|----------------------|---------|
| Age ≤50           | 43 (48.8%)          | 41 (48.8%)           | 0.994   |
| Age >50           | 45 (51.2%)          | 43 (51.2%)           |         |
| Characteristic          | No. (%)         | Value | p-value |
|------------------------|-----------------|-------|---------|
| **Sex**                |                 |       | 0.438   |
| Male                   | 77(87.5%)       | 70(83.3%) |         |
| Female                 | 11(12.5%)       | 14(16.7%) |         |
| **HBV**                |                 |       | 0.928   |
| Yes                    | 81(92.0%)       | 77(91.7%) |         |
| No                     | 7(8.0%)         | 7(8.3%)  |         |
| **AFP**                |                 |       | 0.001   |
| ≥400 ng/ml             | 23(26.1%)       | 42(50%)  |         |
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| **Tumour size**        |                 |       | 0.042   |
| <10cm                  | 75(85.2%)       | 61(72.6%) |         |
| ≥10cm                  | 13(14.8%)       | 23(27.4%) |         |
| **Complete capsule**   |                 |       | 0.888   |
| Yes                    | 15(17.0%)       | 15(17.9%) |         |
| No                     | 73(83.0%)       | 69(82.1%) |         |
| **Microvascular invasion** |             |       | 0.864   |
| Yes                    | 23(26.1%)       | 21(25%)  |         |
| No                     | 65(73.9%)       | 63(75%)  |         |
| **Margin**             |                 |       | 0.004   |
| Clear                  | 85(96.6%)       | 70(83.3%) |         |
| unclear                | 3(3.4%)         | 14(16.7%) |         |
| **Cirrhosis (yes/no)** |                 |       | 0.172   |
| Yes                    | 66(75.0%)       | 55(65.5%) |         |
| No                     | 22(25.0%)       | 29(34.5%) |         |
| **CA19_9**             |                 |       | 0.294   |
| ≥35 U/mL               | 20(22.7%)       | 25(29.8%) |         |
| <35 U/mL               | 68(77.3%)       | 59(70.2%) |         |
| **CEA**                |                 |       | 0.101   |
| ≥5 ng/mL               | 16(18.2%)       | 8(9.5%)   |         |
| <5 ng/mL               | 72(81.8%)       | 76(90.5%) |         |
| **ALB**                |                 |       | 0.341   |
| >35 g/L                | 83(94.3%)       | 76(90.5%) |         |
| ≤35 g/L                | 5(5.7%)         | 8(9.5%)   |         |
| **ALT**                |                 |       | 0.703   |
| >50U/L                 | 29(33.0%)       | 30(35.7%) |         |
| ≤50 U/L                | 59(67.0%)       | 54(64.3%) |         |
| **AST**                |                 |       | 0.903   |
| >40U/L                 | 39(44.3%)       | 38(45.2%) |         |
| ≤40U/L                 | 49(55.7%)       | 46(54.8%) |         |
| TBIL  | 0.084 |
|-----------------|-------|
| >17.1μmol/L     | 28(31.8%) 17(20.2%) |
| ≤17.1μmol/L     | 60(68.2%) 67(79.8%) |

HBV, Hepatitis B virus; AFP, α-fetoprotein; CA19-9, Carbohydrate antigen 19-9; CEA, Carcinoma Embryonic Antigen; ALB, Albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TBIL, Total bilirubin
**Supplementary Table 2 Univariate and Multivariate Cox analysis of factors associate with overall survival of HCC patients in SYSUCC cohort**

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| CA19_9 > 35 U/mL (yes/no)           | 1.349 (0.769-2.367) | 0.296               |                     |                 |
| CEA > 5 ng/mL (yes/no)              | 0.671 (0.287-1.568) | 0.357               |                     |                 |
| Cirrhosis (yes/no)                  | 0.830 (0.471-1.464) | 0.52                |                     |                 |
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| AST > 40U/L (yes/no)                | 2.339 (1.365-4.008) | 0.002               |                     |                 |
| TBIL > 17.1μmol/L (yes/no)          | 1.300 (0.724-2.334) | 0.379               |                     |                 |

HR, Hazard ratio; CI, Confidence interval; HBV, Hepatitis B virus; AFP, α-fetoprotein; CA19-9, Carbohydrate antigen 19-9; CEA, Carcinoma Embryonic Antigen; ALB, Albumin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; TBIL, Total bilirubin

2. CRISPR Knockout Pooled Library.xlsx
This document has been uploaded as a separate file.

3. Ischemia VS Con_Gene_differential_expression.xlsx
This document has been uploaded as a separate file.

4. TACE_ER VS LR_Gene_differential_expression.xlsx
This document has been uploaded as a separate file.

5. P-PLCPRF5-CTR-vs-P-PLCPRF5-sh1.genes.annot
### Antibodies

| Antibodies                  | Company sources          | Identifier       |
|-----------------------------|--------------------------|------------------|
| Rabbit anti-S100A9 (CO-IP)  | Cell signaling technology| Cat # 72590      |
| Rabbit anti-S100A9 (IB)     | Proteintech              | Cat # 26992-1-AP |
| Rabbit anti-S100A9 (IHC, IF)| Abcam                    | Cat # ab63818    |
| Rabbit anti-HIF1A           | Proteintech              | Cat # 20960-1-AP |
| Rabbit anti-DRP1            | Cell signaling technology| Cat # 8570       |
| Rabbit anti-DRP1 (Ser637)   | Cell signaling technology| Cat # 4867       |
| Rabbit anti-PGAM5(IB)       | Cell signaling technology| Cat # 24584      |
| Mouse anti-PGAM5(IHC, IF)   | Santa Cruz               | Cat # sc-515880  |
| Mouse anti-E-cadherin       | BD Transduction          | Cat # 610181     |
| Rabbit anti-N-Cadherin      | Cell signaling technology| Cat # 13116      |
| Rabbit anti-Snail           | Cell signaling technology| Cat # 3879       |
| Rabbit anti-Fibronectin     | Abcam                    | Cat # ab268020   |
| Rabbit anti-Ki67            | Abcam                    | Cat # ab15580    |
| Rabbit anti-HA-Tag          | Cell signaling technology| Cat # 3724       |
| Rabbit anti-His-Tag         | Cell signaling technology| Cat # 12698      |
| Mouse anti-Myc-Tag          | Cell signaling technology| Cat # 2276       |
| Rabbit anti-DYKDDDDK Tag    | Cell signaling technology| Cat # 14793      |
| Rabbit anti-S100A9 (MIF)    | Servicebio               | GB111079         |
| Mouse Anti-EpCAM (MIF)      | Servicebio               | GB14078          |
| Mouse Anti-CD45 (MIF)       | Servicebio               | GB14038          |
| Rabbit Anti-CD68 (MIF)      | Servicebio               | GB113150         |
| Rabbit Anti-CD3 (MIF)       | Servicebio               | GB11014          |
| Rabbit anti-USP10           | Proteintech              | Cat # 19374-1-AP |
| Mouse anti-Alpha Tubulin    | Proteintech              | Cat # 11224-1-AP |
| Mouse anti-Beta Actin       | Proteintech              | Cat # 60008-1-lg |
| Anti-rabbit IgG, HRP-linked | Cell signaling technology| Cat #7074        |
| Anti-mouse IgG, HRP-linked  | Cell signaling technology| Cat #7076        |

### Drugs and chemicals reagents

| Drugs and chemicals reagents | Company sources          | Identifier       |
|-----------------------------|--------------------------|------------------|
| Tasquinimod                 | MedChemExpress           | Cat # HY-10528   |
| ICI 118,551                 | MedChemExpress           | Cat # HY-13951   |
| Atenolol                    | MedChemExpress           | Cat # HY-17498   |
| N-acetyl-L-Cysteine (NAC)   | Beyotime                 | Cat # S0077      |
| MitoSOX                     | Thermo Fisher Scientific | Cat #M36008      |
| Mito tracker red            | Beyotime                 | Cat # C1035      |
| DCFH-DA                     | Beyotime                 | Cat # S0033S     |
| Lipofectamine RNAiMAX       | Invitrogen               | Ca t # 13778030  |
| Item                                      | Supplier                  | Cat #               |
|-------------------------------------------|---------------------------|---------------------|
| Lipofectamine 3000                        | Invitrogen                | L3000015            |
| Mdivi-1                                   | Beyotime                  | SC8028              |
| Mito-tempo                                | MedChemExpress            | HY-112879           |
| Polybrene                                 | ThermoFisher              | 107689              |
| Zombie NIR™ Fixable Viability Kit         | Biolegend                 | 423105              |
| Cell Lysis Buffer                         | Cell signaling technology | 9803S               |
| Trizol reagent                            | Invitrogen                | 15596018            |
| Pierce IP lysis buffer                    | Invitrogen                | 87787               |
| Protein A/G PLUS-Agarose beads            | Santa Cruz                | sc-2003             |
| Puromycin                                 | Invitrogen                | A1113802            |
| Seahorse XFe24 FluxPak                    | Seahorse bioscience       | 102340-100          |
| Seahorse XF Cell Mito Stress Test kit     | Seahorse bioscience       | 103015-100          |
| Seahorse XF Glycolysis Stress Test kit    | Seahorse bioscience       | 103020-100          |
| SimpleChIP® Enzymatic Chromatin IP Kit    | Cell signaling technology | 9003                |
| (Magnetic Beads)                          |                           |                     |
| Migration assay                           | Corning                   | 354578              |
| Cell counting kit                         | Dojindo                   | CK04                |
| S100A9 ELISA Kit                          | CUSABIO                   | CSB-E11834h          |
| Lentil-Pac HIV Expression Packaging Kit   | GeneCopoeia               | LT002               |
| RNA Quick Purification kit                | ESscience                 | RN001               |
| ATP Colorimetric/Fluorometric Assay Kit   | BioVision                 | K354-100            |
| Luc-Pair™ Duo-Luciferase HS Assay Kit     | GeneCopoeia               | LF005               |
| Sg Negative control                       | Kidan Biosciences         | NA                  |
| GACCGGGGGGAGGAGCTGTTCACCG                  |                           |                     |
| S100A9-sgRNA #1                           | Kidan Biosciences         | NA                  |
| GACTTGGCAAAATGTGCGAGC                     |                           |                     |
| S100A9-sgRNA #2                           | Kidan Biosciences         | NA                  |
| GCACCCAGACACCCTGAACC                      |                           |                     |
| S100A9-shRNA #2                           | Genepharma                | NA                  |
| GGCCAAATAAAAGTCTTCTTCT                    |                           |                     |
| S100A9-shRNA #4                           | Genepharma                | NA                  |
| GCCTGTATGTCAACTGCT                       |                           |                     |
| si PGAM5                                  | Genepharma                | NA                  |
| GUCCUUUAUUUUUGUCATT                       |                           |                     |
| si USP10 #1                               | Genepharma                | NA                  |
| GCUUUGGAGGAGAGUUCUATT                     |                           |                     |
| si USP10 #2                               | Genepharma                | NA                  |
| GCACACCAGGAAGCAUATT                       |                           |                     |

**Primer sequences**

| Construct  | Direction | Sequence (5’ - 3’)               |
|------------|-----------|-----------------------------------|
| S100A9 (Human) | Forward  | GCACCCAGACACCCTGAACCA |
| Gene          | Primer 1 | Primer 2 |
|--------------|----------|----------|
| hS100A9-chip-site A | Forward  | Reverse  |
| hS100A9-chip-site A | Reverse  | Forward  |
| hS100A9-chip-site B | Forward  | Reverse  |
| hS100A9-chip-site B | Reverse  | Forward  |
| hS100A9-chip-site C | Forward  | Reverse  |
| Mouse        | Forward  | Reverse  |
| Actin        | Forward  | Reverse  |
| USP10        | Forward  | Reverse  |
| NOX1        | Forward  | Reverse  |
| NOX2        | Forward  | Reverse  |
| NOX4        | Forward  | Reverse  |
| NOX5        | Forward  | Reverse  |
| DUOX1       | Forward  | Reverse  |
| DUOX2       | Forward  | Reverse  |
References

1. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen T, Heckl D, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 2014;343:84-87.

2. Cai MY, Tong ZT, Zheng F, Liao YJ, Wang Y, Rao HL, Chen YC, et al. EZH2 protein: a promising immunomarker for the detection of hepatocellular carcinomas in liver needle biopsies. Gut 2011;60:967-976.

3. Wang C, Liao Y, He W, Zhang H, Zuo D, Liu W, Yang Z, et al. Elafin promotes tumour metastasis and attenuates the anti-metastatic effects of erlotinib via binding to EGFR in hepatocellular carcinoma. J Exp Clin Cancer Res 2021;40:113.