Identification and Functional Characterization of Long Non-coding RNA

**MIR22HG as a Tumor Suppressor for Hepatocellular Carcinoma**

Dong-Yan Zhang\(^1,2\),†, Xue-Jing Zou\(^2,3\),†, Chuan-Hui Cao\(^1,2\), Ting Zhang\(^2,4\), Ling Lei\(^1,2\), Xiao-Long Qi, Li Liu\(^2,3,4\)* and De-Hua Wu\(^1,2\)*

**Supplementary materials and methods**

**qRT-PCR**

TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) was used for the extraction of total RNAs. First-strand cDNA was synthesized by the PrimeScript\(^\text{™}\) 1st Strand cDNA Synthesis Kit (TaKaRa, Tokyo, Japan). Real-time PCR was carried out using SYBR® Green PCR kit (TaKaRa). Primers were shown in Supplementary Table 5.

**Plasmid construction and transient transfection**

Wild type HMGB1 3′-UTR sequence (containing miR-22-3p binding site) and mutant HMGB1 3′-UTR sequence (miR-22-3p binding site mutation) were amplified and inserted into psiCHECKTM-2 Vector (Promega, Madison, WI, USA) to construct plasmids used in the following dual-luciferase reporter assays. The open reading frame (ORF) of HMGB1 was cloned and inserted into pCMV6-AC-GFP (Origene, Rockville, MD, USA) vector to generate HMGB1. The primer sequences were listed in Supplementary Table 5. Cells seeded overnight were transiently transfected with
plasmids or control vectors at 90% cell confluency using Lipofectamine 2000 (Invitrogen). 48 hours later, cells were harvested for following assays.

**Lentiviral construction and transduction**

The lentiviral vector containing full-length of human *MIR22HG* gene sequence was achieved from Genechem Company Ltd (Shanghai, China). Lentiviral vector containing full-length of human *MIR22HG* gene sequence or the empty lentiviral vector were introduced to SK-Hep-1 and SMMC-7721 cells according to the manufacture’s instruction. To select clones stably overexpressing *MIR22HG*, cells after transfection were exposed to puromycin for 2 weeks. 2 weeks later, cells were harvested for RNA isolation. The expression of *MIR22HG* was then determined using qRT-PCR.

**Cell counting kit-8 (CCK-8) assays**

Cellular proliferation capacity was tested with Cell Counting Kit 8 (CCK-8) assay (Dojindo, Kumamoto, Japan) following the manufacturer’s protocol. $1 \times 10^3$ cells per well were seeded in 96-well plate and grow for the given time points. 24 hours later, 10μl CCK-8 reagent was added to each plate, and cultured for another 2 hours at 37 °C. Then optical density was test at 450nm. All experiments were performed in triplicate.

**5-ethynyl-20-deoxyuridine (EdU) incorporation assays**
SMMC-7721 cells seeded in 24-well plates were cultured in humidified incubator for 12h. Cells were transiently transfected with vectors or siRNAs 12h later according to the protocol. EdU Cell Proliferation Assay Kit (Ribobio, Wuhan, China) were used to detect the cellular proliferation after 48h. Briefly, fixed SMMC-7721 cells were stained with EdU following the recommendations after incubation with 50μM EdU. The ratio of EdU positive cells to total Hoechst positive cells was regarded as EdU incorporation rate.

miRNA transfection

The miR-22-3p mimic and miR-22-3p inhibitor were obtained from Shanghai GenePharma Company, China. The miRNA mimics or inhibitor were introduced into HCC cells using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen) following the manufacturer’s instruction. After incubation for 24-48 hours, cells were ready for the following assays.

Luciferase reporter assays

SMMC-7721 cells were cultured in 24-well plates and co-transfected with 40ng plasmids containing wild type HMGB1 3’-UTR sequences (termed psiCHECK-wt-HMGB1) or plasmids containing mutant HMGB1 3’-UTR sequences (termed psiCHECK-mut-HMGB1) and 20 pmol miR-22-3p mimic or negative control. Luciferase activities of both firefly and Renilla were examined using the Dual-Luciferase® Reporter Assay System ((Promega) 48 hours later.
RNA interference

Small interfering RNAs were used in the current study to silence human *MIR22HG* gene expression. All target sequences for *MIR22HG* were synthesized by GenePharma Company and listed in Supplementary Table 6. Lipofectamine RNAiMAX Transfection Reagent (Invitrogen) was used to perform transfection assays.

*In situ* hybridization (ISH)

ISH was carried out with the ISH Kit (Boster Bio-Engineering Company, Wuhan, China) to explore the expression of *MIR22HG* in HCC tissue and matched non-tumor tissues. Two pathologists who blinded to the clinical parameters reviewed and scored the ISH-staining regions for *MIR22HG*. The staining intensity was scored as follows: 0 (negative), 1 (weak), 2 (medium), 3 (strong). The score of staining extent was as follows: 0 (10%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%). The total score for *MIR22HG* was calculated based on the intensity and extent scores, ranging from 0 to 7. A total score ≥4 was defined as belonging in the high-expression group.

Immunohistochemistry (IHC)

IHC staining was performed to determine the expression of Ki-67 and HMGB1 in tumor tissues from subcutaneous xenograft model using Dako Envision System (Dako, Carpinteria, CA, USA) according to the manufacturer’s recommended protocol. The
tumor tissues were fixed with 10% formalin, embedded in paraffin, and then sectioned 4 μm in thickness. After baking at 65°C for 2 hours, sections were deparaffinized and rehydrated. Sections were submerged in sodium citrate buffer (pH 6.0) for antigen retrieval. After incubation with 0.3% H₂O₂ for 15 min to block the endogenous peroxidase, the sections were incubated with antibody for Ki-67 or HMGB1 overnight at 4°C. Sections were incubated with peroxidase labeled polymer conjugated to a secondary antibody at room temperature for 50 minutes after washing. Finally, diaminobenzidine (DAB) was used for color reactions.

**Western blot**

Total protein was extracted using RIPA buffer (Cell signaling Technology, Boston, MA, USA). The protein lysates were separated on a 12% SDS–polyacrylamide gel, and transferred onto polyvinylidene fluoride (Millipore, Bedford, MA, USA) membranes. After blocking with 5% BSA for 1 hour at room temperature, the membranes were incubated with primary antibodies overnight at 4°C. After washing, the membranes were incubated with the corresponding secondary antibodies conjugated to horseradish peroxidase. The membrane signals were detected using commercial ECL kit (Pierce, Rockford, IL, USA). Image J program was used to analyze the band intensity of western blotting and the normalization. The primary antibodies were shown in Supplementary Table 7.

**RNA-Binding Protein Immunoprecipitation (RIP) analyses**
RIP assay was carried out following recommendations of the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA). In brief, magnetic beads were pre-incubated with an anti-HuR antibody or anti-mouse IgG for about 30 minutes at room temperature. 30 minutes later, magnetic beads were washed with RIP wash buffer. After washing, cell lysates were immunoprecipitated with magnetic beads at 4°C overnight. After immunoprecipitation, magnetic beads were washed with RIP wash buffer for 5 times. RNA which bound to beads was then purified from RNA-protein complexes, and analyzed using qRT-PCR.

**RNA Pull-Down Assays**

RNAs were *in vitro* transcribed using T7 RNA polymerase (Thermo scientific Transcript Aid T7 High Yield Transcription Kit, Waltham, MA) and biotin-labeled using Pierce™ RNA 3’ End Desthiobiotiny Kit (Thermo Fisher scientific). RNA pull-down assay was performed following the instructions of the Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher scientific). Magnetic Beads were subjected to RNA (50 pmol) capture in RNA capture buffer (20mM Tris-HCl pH 7.5, 1M NaCl, 1mM EDTA) for 15-30 min at room temperature under agitation. The RNA-captured beads were washed once with 50μl 20mM Tris (pH 7.5) and incubated with 2 mg SMMC-7721 cell lysates for 30-60 min at 4°C under rotation. The RNA-binding protein complexes were then washed twice with wash buffer (20mM Tris-HCl pH 7.5, 10mM NaCl, 0.1% Tween-20 Detergent) and eluted with Biotin Elution Buffer.
**Immunofluorescence assay**

SMMC-7721 cells transfected with *MIR22HG* and *MIR22HG*-mut were seeded on coverslips. 24h later, cells on coverslips were washed with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 15 minutes, and then permeabilized with 0.25% Triton for 10 minutes. After that, cells were incubated with anti-HuR antibody at 4°C overnight. Cells were incubated with rhodamine-conjugated goat antibodies against mouse IgG (Abcam, Cambridge, UK) after washing with PBS for 5 times. Nuclei was stained with DAPI. A confocal laser scanning microscope (FV1000; Olympus, Center Valley, PA) were used to image the coverslips.
Supplementary Fig. 1. *MIR22HG* overexpression inhibits tumor growth and metastasis *in vivo.* (A) Schematic presentation of the *MIR22HG* transcripts. The primer pairs used to detect different variants of *MIR22HG* by RT-qPCR are indicated by arrows. (B) The expression levels of different variants of *MIR22HG* were detected.
in 7 paired HCC tumor tissues and non-tumor tissues using RT-qPCR. Variant 1 was the most abundant isoform in non-tumor tissues but dramatically downregulated in tumor tissues, as its expression value peaked nearly 20 in non-tumor tissue but bottomed only 3.9 in tumor tissue. (C) MIR22HG overexpression in SK-Hep-1 cells inhibited tumor growth in vivo. The volume and weight of subcutaneous xenograft tumors were significantly different between the MIR22HG and control groups (n = 6). *P< 0.05. (D) H&E-stained sections of xenograft tumors from the MIR22HG and control groups. (E) IHC staining for Ki-67 of xenograft tumors from the MIR22HG and control groups. (F) Representative microscopic images of H&E-stained pulmonary metastasis in the MIR22HG-overexpressing and control groups. Lung metastasis in both groups were quantified.*P< 0.05.
Supplementary Fig. 2. Knockdown of MIR22HG promotes tumor growth both in vitro and in vivo. (A) Knockdown efficiency of 2 different shRNAs specific to MIR22HG was examined in HCC-LM3 cells by qRT-PCR, ***P < 0.0001. (B) MIR22HG deletion in HCC-LM3 cells promoted cellular proliferative capacity, **P < 0.01. (C) MIR22HG deletion in HCC-LM3 cells promoted tumor growth in vivo. HCC-LM3 cells with sh-MIR22HG (right side) and control vector (left side) were injected into the bilateral flanks of the nude mice. The volume and weight of subcutaneous xenograft tumors were significantly different between the sh-MIR22HG and control groups (n = 10). *P < 0.05. (D) Left panel: H&E-stained sections of xenograft tumors from sh-MIR22HG and control groups. Right panel: IHC staining
for Ki-67 of xenograft tumors from the sh-MIR22HG and control groups.

Supplementary Fig. 3. Knock down of MIR22HG promotes tumor invasion and metastasis both in vitro and in vivo. (A) Motility and invasive ability of HCC-LM3 cells after transfection of 2 different shRNAs specific to MIR22HG were evaluated by in vitro transwell assays. (B) sh-MIR22HG-HCC-LM3 cells and control cells were injected intravenously into mice and bioluminescence images were obtained (n = 10). Left panel: Representative images of pulmonary colonization at 3 weeks after injection. Right panel: Numbers of mice with lung metastasis in both groups. (C) Representative microscopic images of H&E-stained pulmonary metastasis in the sh-MIR22HG and control groups. Lung metastasis in both groups were quantified.
Supplementary Fig. 4. miR-22-3p inhibits cell migration and invasion. (A) miR-22-3p expression was detected in MIR22HG knockdown HCC-LM3 cells. *P<0.05; **P < 0.01; ***P< 0.0001. (B) Expression of miR-22-3p was examined in SK-Hep-1 and SMMC-7721 cells transfected with miR-22-3p mimics (left panel) or miR-22-3p inhibitor (right panel). (C) Effects of miR-22-3p overexpression on
migration and invasion were detected in SK-Hep-1 and SMMC-7721 cells. (D)

Effects of miR-22-3p inhibition on migration and invasion were detected in SK-Hep-1
and SMMC-7721 cells.

Supplementary Fig. 5. Up-regulation of miR-22-3p suppresses the promotion of
migration and invasion by MIR22HG knockdown in HCC-LM3 cells. (A)

Expression of MIR22HG and miR-22-3p were detected in the indicated conditions.

**P < 0.01, ***P < 0.0001, ns: not significant. (B) Representative images of migration
and invasion assays of the indicated cell lines. Silencing of MIR22HG promoted migration and invasion in HCC-LM3 cells, and these effects were destroyed by miR-22-3p mimics. (C) Quantification of cell migration and invasion in the indicated cell lines. Each bar represents the mean ± SEM of three independent experiments.

***P < 0.0001, ns: not significant.
Supplementary Fig. 6. HMGB1 is regulated by MIR22HG in HCC. (A) miR-22-3p expression in SK-Hep-1 cells after transfection with miR-22-3p mimics. (B) mRNA expression of the indicted genes in SK-Hep-1 cells after transfection with miR-22-3p mimics. The mRNA expression of CD147, HMGB1, SP1, MYCBP, and TIAM1 did not alter after overexpression of miR-22-3p. (C) mRNA expression of the indicated genes in SK-Hep-1 and SMMC-7721 cells after transfection with MIR22HG. The mRNA expression of the indicated genes remained stable in spite of MIR22HG overexpression both in SK-Hep-1 and SMMC-7721 cells. (D) Correlation between miR-22-3p expression and mRNA expression of the indicated genes in 14 HCC tissues. The mRNA expression of the indicated genes did not correlate with miR-22-3p in 14 HCC tissues as measured by qRT-PCR. (E) Protein expression levels of HMGB1 in the indicated cell lines as determined by western blotting. The promotion effect of si-MIR22HG on HMGB1 expression was abolished by miR-22-3p mimics. (F) Expression of HMGB1 and its downstream effectors in SK-Hep-1 cell line as determined by western blotting. (G) HMGB1 expression in xenograft tumors developed by injecting indicated cells into nude mice as detected by IHC. The expression of HMGB1 was down-regulated in MIR22HG-overexpressing xenograft tumors.
Supplementary Fig. 7. MIR22HG-mut inhibits cellular proliferation, migration, invasion of HCC cells. (A) Ectopic expression of MIR22HG induced miR-22-3p expression, whereas, ectopic expression of MIR22HG with miR-22-3p region deletion mutation (MIR22HG-mut) did not influenced the expression of miR-22-3p. ***P <
0.0001, ns: not significant. (B) Overexpression of MIR22HG and MIR22HG-mut significantly inhibited cellular proliferation as detected by CCK-8 assay. ***$p < 0.0001$. (C) *In vitro* migration and invasion assay showed that both MIR22HG and MIR22HG-mut repressed HCC cell migration and invasion. **$p < 0.01$, ***$p < 0.0001$. (D) Fractionation of SMMC-7721 cells followed by qRT-PCR indicated that MIR22HG mainly localized in cytoplasm. U6 RNA served as a positive control for nuclear gene expression, and GAPDH RNA served as a positive control for cytoplasm gene expression. (E) The expression level of MIR22HG was examined in SMMC-7721 cells after transfection with HuR specific siRNAs. ***$p < 0.0001$. (F) Efficiency of HuR overexpression or silencing was evaluated by western blotting. (G-H) Correlation between HuR mRNA expression and MIR22HG expression level was detected in HCC tissues from 52-patient cohort (G) and GSE14520 (H), respectively. (I) HuR protein expression level was detected in 13 paired HCC tissues and non-tumor tissues from 52-patient cohort. (J) Correlation between HuR protein expression and MIR22HG expression level was analyzed.
Supplementary Fig. 8. MIR22HG or MIR22HG-mut overexpression alters the subcellular localization of HuR.

(A) The translocation of HuR from the cytoplasm to the nucleus was detected by immunofluorescence staining after MIR22HG or MIR22HG-mut overexpression in SMMC-7721 cells. (B) Western blotting showed that HuR translocated from cytoplasm to the nucleus after MIR22HG or MIR22HG-mut overexpression in SMMC-7721. (C) The translocation of HuR from the nucleus to the cytoplasm was detected by immunofluorescence staining after MIR22HG deletion in HCC-LM3 cells. (D) Western blotting showed that HuR translocated from the nucleus to the cytoplasm after silencing MIR22HG in HCC-LM3 cells.
Supplementary Fig. 9. Silencing MIR22HG increases expression of the target genes of HuR. (A) Effect of MIR22HG knockdown on the mRNA expression of the indicated genes. (B) MIR22HG knockdown increased the expression of the indicated proteins. (C-D) The mRNA (C) and protein (D) expression of the indicated genes was detected in SMMC-7721 cells transfected with an miR-22-3p mimic and inhibitor.
Supplementary Fig. 10. MIR22HG induces miR-22-3p to restrain HMGB1 translation and interacts with HuR, thus interrupting HuR binding to its target mRNAs.

In HCC cells, at the absence of MIR22HG, cytoplasmic HuR is able to stabilize or promote the translation of its target mRNAs including CTNNB1, CCNB1, BCL2, COX2, and C-FOS. The upregulation of these oncogenes results in tumor progression. Low level of MIR22HG also undergoes unstabilization due to its insufficient
interaction with HuR. When \textit{MIR22HG} increases, it acts as a tumor suppressor in two aspects. Firstly, it generates pre-miR-22, the precursor for miR-22-3p which inhibits mRNA HMGB1 translation. Secondly, lncRNA \textit{MIR22HG} competively binds to HuR in cytoplasm, leading to the stabilization of \textit{MIR22HG} and promoting the nuclear translocation of HuR; therefore, the expressions of the aforementioned HuR target mRNAs are restrained by \textit{MIR22HG}-HuR interaction. In all, the presence of \textit{MIR22HG} reduces the level of \textit{CTNNB1, CCNB1, BCL2, COX2, C-FOS} and \textit{HMGB1}, and hence restricts HCC progression.

**Supplementary Tables**

**Supplementary Table 1**
Clinicopathological characteristics of 52 HCC patients (52-patient cohort)

| Feature                        | N (%)       |
|--------------------------------|-------------|
| Age (years)                    |             |
| \(\leq 55\)                    | 36 (69.2)   |
| >55                            | 16 (30.8)   |
| Gender                         |             |
| Male                           | 45 (86.5)   |
| Female                         | 7 (13.5)    |
| Tumour size (cm)               |             |
| \(\leq 5\)                     | 22 (42.3)   |
| >5                             | 30 (57.7)   |
| Edmondson Grade                |             |
| I-II                           | 13 (25.0)   |
| III-IV                         | 39 (75.0)   |
| BCLC stage                     |             |
| A                              | 30 (57.7)   |
| B+C+D                          | 22 (42.3)   |
| Liver cirrhosis                |             |
| Yes                            | 38 (73.1)   |
| No                             | 14 (26.9)   |
| Portal vein tumour thrombus    |             |
|                |        |        |
|----------------|--------|--------|
| **No. tumour**  | **Yes**| 15 (28.8) |
|                | **No** | 37 (71.2)  |
| **Solitary**   | 42 (80.8) |
| **Multiple**   | 10 (19.2) |
| **HBV**        |        |        |
| **With**       | 47 (90.4) |
| **Without**    | 5 (9.6)   |
**Supplementary Table S2**

Clinicopathological characteristics of 145 HCC patients (145-patient cohort)

| Feature                                      | N (%)  |
|----------------------------------------------|--------|
| Age (years)                                  |        |
| ≤55                                          | 76 (52.4) |
| >55                                          | 69 (47.6) |
| Gender                                       |        |
| Male                                         | 121 (83.4) |
| Female                                       | 24 (16.6) |
| Tumour size (cm)                             |        |
| ≤5                                          | 65 (44.8) |
| >5                                          | 80 (55.2) |
| Edmondson grade                              |        |
| I-II                                         | 118 (81.4) |
| III-IV                                       | 27 (18.6) |
| BCLC stage                                   |        |
| A                                            | 103 (71.0) |
| B+C+D                                        | 42 (29.0) |
| Portal vein tumour thrombus                  |        |
| No                                           | 125 (86.2) |
| Yes                                          | 20 (13.8) |
| Liver cirrhosis                              |        |
| Yes                                          | 107 (73.8) |
| No                                           | 38 (26.2) |
| Metastasis                                   |        |
| Yes                                          | 6 (4.1) |
| No                                           | 139 (95.9) |
| Relapse                                      |        |
| Yes                                          | 48 (33.1) |
| No                                           | 97 (66.9) |
| No. tumour                                   |        |
| Solitary                                     | 116 (80.0) |
| Multiple                                     | 29 (20.0) |
| HBV                                          |        |
| With                                         | 124 (85.5) |
| Without                                      | 21 (14.5) |
**Supplementary Table 3**

Correlation between *MIR22HG* expression and HCC clinicopathologic features in 145-patient cohort

| MIR22HG expression levels |  |  |
|---------------------------|---|---|
| high expression | low expression |  |
| Gender |  |  |
| Male | 48 | 73 |
| Female | 9 | 15 |
| Age (years) |  |  |
| ≤55 | 22 | 54 |
| >55 | 35 | 34 |
| Edmondson Grade |  |  |
| I-II | 53 | 65 |
| III-IV | 5 | 22 |
| Liver cirrhosis |  |  |
| With | 42 | 65 |
| without | 15 | 23 |
| HBV |  |  |
| With | 51 | 73 |
| Without | 6 | 15 |
| Portal vein tumour thrombus |  |  |
| No | 55 | 70 |
| Yes | 2 | 18 |
| No. tumour |  |  |
| Solitary | 50 | 66 |
| Multiple | 7 | 22 |
| Tumour size (cm) |  |  |
| ≤5 | 29 | 36 |
| >5 | 28 | 52 |
| BCLC stage |  |  |
| A | 48 | 55 |
| B+C+D | 9 | 33 |
| Relapse |  |  |
| Yes | 15 | 33 |
| No | 42 | 55 |
| Metastasis |  |  |
| Yes | 3 | 3 |
| No | 54 | 85 |

Abbreviations: AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer. *The values had statistically significant differences.
Supplementary Table S4

Overlapping genes predicted by miRanda and picTar

| miRNA           | GeneName   |
|-----------------|------------|
| has-miR-22-3p   | JARID2     |
| has-miR-22-3p   | PTPN1      |
| has-miR-22-3p   | CTSC       |
| has-miR-22-3p   | COPS7B     |
| has-miR-22-3p   | ELOVL6     |
| has-miR-22-3p   | IKZF4      |
| has-miR-22-3p   | GALNT3     |
| has-miR-22-3p   | SEMA6D     |
| has-miR-22-3p   | SIRT1      |
| has-miR-22-3p   | BIN1       |
| has-miR-22-3p   | CDKN1A     |
| has-miR-22-3p   | PIP4K2B    |
| has-miR-22-3p   | EDC3       |
| has-miR-22-3p   | SMG7       |
| has-miR-22-3p   | CALM3      |
| has-miR-22-3p   | STYX       |
| has-miR-22-3p   | CAMK2N1    |
| has-miR-22-3p   | MXD1       |
| has-miR-22-3p   | HOXA4      |
| has-miR-22-3p   | KDM3A      |
| has-miR-22-3p   | ZBTB39     |
| has-miR-22-3p   | PRPF38A    |
| has-miR-22-3p   | BCL9       |
| has-miR-22-3p   | EP300      |
| has-miR-22-3p   | PURB       |
| has-miR-22-3p   | FRAT2      |
| has-miR-22-3p   | IPO7       |
| has-miR-22-3p   | LRRRC16A   |
| has-miR-22-3p   | NFYA       |
| has-miR-22-3p   | ERBB3      |
| has-miR-22-3p   | EMILIN3    |
| has-miR-22-3p   | TRUB1      |
| has-miR-22-3p   | FAM96A     |
| has-miR-22-3p   | BATF3      |
| has-miR-22-3p   | C15orf29   |
| has-miR-22-3p | NCOA1          |
|-------------|----------------|
| has-miR-22-3p | MAPK14         |
| has-miR-22-3p | SRF            |
| has-miR-22-3p | MYCBP          |
| has-miR-22-3p | FBXO45         |
| has-miR-22-3p | CD147          |
| has-miR-22-3p | KDM6B          |
| has-miR-22-3p | PHF8           |
| has-miR-22-3p | ERGIC2         |
| has-miR-22-3p | SV2A           |
| has-miR-22-3p | NET1           |
| has-miR-22-3p | DPF2           |
| has-miR-22-3p | LRP12          |
| has-miR-22-3p | BTBD10         |
| has-miR-22-3p | WASF1          |
| has-miR-22-3p | NR3C1          |
| has-miR-22-3p | CTDSPL2        |
| has-miR-22-3p | RTN3           |
| has-miR-22-3p | UNK            |
| has-miR-22-3p | SATB2          |
| has-miR-22-3p | PPP1R15B       |
| has-miR-22-3p | CCDC47         |
| has-miR-22-3p | DPYSL3         |
| has-miR-22-3p | STK39          |
| has-miR-22-3p | FBXL19         |
| has-miR-22-3p | MTF2           |
| has-miR-22-3p | TIAM1          |
| has-miR-22-3p | WRNIP1         |
| has-miR-22-3p | BTG1           |
| has-miR-22-3p | NRAS           |
| has-miR-22-3p | SLC2A1         |
| has-miR-22-3p | LRRRC1         |
| has-miR-22-3p | HERPUD2        |
| has-miR-22-3p | FBXW7          |
| has-miR-22-3p | LIN7C          |
| has-miR-22-3p | ATXN7          |
| has-miR-22-3p | UBE2K          |
| has-miR-22-3p | HSPG2          |
| has-miR-22-3p | CENPV          |
| has-miR-22-3p | EPC1           |
| has-miR-22-3p | LGALS1         |
| has-miR-22-3p | STAG2         |
|--------------|--------------|
| has-miR-22-3p | CYTH3        |
| has-miR-22-3p | NAA20        |
| has-miR-22-3p | DPY30        |
| has-miR-22-3p | STX4         |
| has-miR-22-3p | HMGB1        |
| has-miR-22-3p | ITGB3BP      |
| has-miR-22-3p | EFNA5        |
| has-miR-22-3p | AKT3         |
| has-miR-22-3p | MAT2A        |
| has-miR-22-3p | CPEB1        |
| has-miR-22-3p | GNB4         |
| has-miR-22-3p | ZNRF2        |
| has-miR-22-3p | CLDND1       |
| has-miR-22-3p | RAB5B        |
| has-miR-22-3p | HNRNPU1L2    |
| has-miR-22-3p | PDK1L        |
| has-miR-22-3p | MSL2         |
| has-miR-22-3p | SP1          |
| has-miR-22-3p | TCF7L2       |
| has-miR-22-3p | PLCXD3       |
| has-miR-22-3p | POGK         |
| has-miR-22-3p | CHD7         |
| has-miR-22-3p | DNM3         |
| has-miR-22-3p | TP53INP1     |
| has-miR-22-3p | FOXP1        |
| has-miR-22-3p | C6orf62      |
| has-miR-22-3p | NDEL1        |
| has-miR-22-3p | MAX          |
| has-miR-22-3p | SNRK         |
| has-miR-22-3p | PLAGL2       |
| has-miR-22-3p | FOSL1        |
| has-miR-22-3p | RNF38        |
| has-miR-22-3p | ETS2         |
| has-miR-22-3p | FAM49B       |
| has-miR-22-3p | RSBN1        |
| has-miR-22-3p | TGFBR1       |
| has-miR-22-3p | MACROD2      |
| has-miR-22-3p | MAP2K4       |
| has-miR-22-3p | C17orf58     |
| has-miR-22-3p | ARFIP2       |
| Gene   | sequence                                      |
|--------|-----------------------------------------------|
| MIR22HG (Variant1)-F | 5′ ATCCAAAGCAGGACAGCA 3′                |
| MIR22HG (Variant1)-R | 5′ TGGCAGGGTTACACTCACT 3′                |
| MIR22HG (Variant2)-F | 5′ CCAGCTAAAGCTGCCAGTTG 3′                |
| MIR22HG (Variant2)-R | 5′ CAGACACAGCTTCCTGGGT 3′                |
| MIR22HG (Variant3)-F | 5′ ACATTTCTGGACCTGAGGAGC 3′               |
| MIR22HG (Variant3)-R | 5′ GGGCAAAGGCTCTCAACTT 3′                |
| MIR22HG (Variant4)-F | 5′ CGAACACAGGGTGGATGAT 3′                |
| MIR22HG (Variant4)-R | 5′ CGCACATATGGTCCACATCT 3′               |
| HMGB1-F | 5′ CGCTTTTTGATGGAGTGCTG 3′                |
| HMGB1-R | 5′ AAGGGGAAAAACTTTGCCATCCCT 3′             |
| MYCBP-F | 5′ CCATTACAAGCGCCGACTC 3′                |
| MYCBP-R | 5′ CACTGTTAGGTTCCTGGGTCTTC 3′             |
| SP1-F | 5′ AATTTGCCTGCCCTGAGTGC 3′                |
| SP1-R | 5′ TTGGACCCCATGCTACCTTG 3′                |
| CD147-F | 5′ TCGCGCTGCTGGGCACC 3′                |
| CD147-R | 5′ TGGCGCTGCTTAGCGCCACC 3′              |
| TIAM1-F | 5′ GCATTCCTCTAGA ATCAGCAGG 3′           |
| TIAM1-R | 5′ TGCGAAAGGCAATTCTCAG 3′              |
| CTNNB1-F | 5′ ACAGGAAGACATCAGGAGC 3′            |
| CTNNB1-R | 5′ CAGTGGGATGGTGGGTGTAAGA 3′            |
| CCNB1-F | 5′ GCATTCCTCGGAGAGCAT 3′             |
| CCNB1-R | 5′ TGTAGAGTTGGTGACATCAG 3′            |
| Gene       | Primer 5' Sequence | Primer 3' Sequence |
|------------|--------------------|--------------------|
| BCL2-F     | 5’ GAACTGGGGGAGGATTGTGG 3’ |
| BCL2-R     | 5’ ACAAAAGGCAATCCCAGCCTC3’ |
| HIF1A-F    | 5’ TCTGGATGCCTGGATTGG 3’ |
| HIF1A-R    | 5’ GCACCAAGAGGCTGTATGT 3’ |
| COX2-F     | 5’ CCGGGAATACTGCACCTAT 3’ |
| COX2-R     | 5’ CGCGCTCGACCCATACAG 3’ |
| MDM2-F     | 5’ CGAGCCTTGGCTGTCTCTT 3’ |
| MDM2-R     | 5’ ACATTTGCTGCTCTCTCAC3’ |
| VEGFA-F    | 5’ CGCAAGAAAATCCCGGTATAA 3’ |
| VEGFA-R    | 5’ TCTCCGCTCTGAGCAAGG 3’ |
| C-FOS-F    | 5’ GGGGCAAGGTTGGAACAGTTAT 3’ |
| C-FOS-R    | 5’ AGGATGCCGCTTTCTCCCA 3’ |
| HuR-F      | 5’ AGAGATTCAGGTCTTCTCCC 3’ |
| HuR-R      | 5’ CCTGCCGCCAGTGTAGATG 3’ |
| miR-22-3p-RT | 5’CTCAACTTGTTGCTGGAGTGGCAATTC AGTTGAGACAGTT 3’ |
| miR-22-3p-F | 5’ACACTCCAGCTGGGAAGCTGCAAGTGA AG 3’ |
| U6-F       | 5’CTGGCTCGGCAGCACA 3’ |
| U6-R       | 5’ AACGCTTCAAGATTTGCGT 3’ |
| psiCHECK-HMGB1-wt-F | 5’CCGCTCGAGCCGACTAAACCTTGCTGT 3’ |
| psiCHECK-HMGB1-wt-R | AAAGCTTTTATTAGC 3’ |
**Supplementary Table S6**

Small-interfering RNA sequences used in this study

| siRNA          | sense sequence         | anti-sense sequence                  |
|----------------|------------------------|--------------------------------------|
| si-MIR22HG-1   | 5’ cccaagguaguuggucuutt 3’ | 5’ aagaccaacuaaccuugggtt 3’          |
| si-MIR22HG-2   | 5’ gcccagecuaguuguuuatt 3’ | 5’ uaaacauuacaggcuggettt            |
| si-MIR22HG-3   | 5’ ggcuuuccggaugagcuutt 3’ | 5’ aacuguauccaggaagcttt            |
**Supplementary Table S7**

Information on antibodies used in this study

| Antibody           | WB  | IHC | IF  | Specificity      | Company                      |
|--------------------|-----|-----|-----|-----------------|------------------------------|
| HMGB1 (ab79823)    | 1:1000 |     | 1:200 | Mouse monoclonal | Abcam                        |
| CD147 (BS6536)     | 1:1000 |     |     | Rabbit polyclonal | Bioworld Technology         |
| β-actin (sc-8432)  | 1:2000 |     |     | Mouse monoclonal | Santa Cruz Biotechnology     |
| TIAM1 (ab70225)    | 1:1000 |     |     | Rabbit polyclonal | Abcam                        |
| SP1(BS3622)        | 1:1000 |     |     | Rabbit polyclonal | Bioworld Technology         |
| MYCBP (ab66331)    | 1:1000 |     |     | Rabbit polyclonal | Abcam                        |
| MMP9 (sc-10737)    | 1:1000 |     |     | Rabbit polyclonal | Santa Cruz Biotechnology     |
| ERK1/2 (#9102)     | 1:1000 |     |     | Rabbit polyclonal | Cell Signaling Technology   |
| p-ERK1/2 (#4370)   | 1:1000 |     |     | Rabbit polyclonal | Cell Signaling Technology   |
| Ki-67 (BS1454)     |     | 1:100 |     | Rabbit polyclonal | Bioworld Technology         |
| HuR (SC5261)       | 1:1000 |     | 1:100 | Mouse monoclonal | Santa Cruz Biotechnology     |
| β-catenin (ab32572) | 1:1000 |     |     | Rabbit polyclonal | Abcam                        |
| CyclinB1 (BS1392)  | 1:1000 |     |     | Rabbit polyclonal | Bioworld Technology         |
| Bcl2 (1017-1)      | 1:1000 |     |     | Rabbit polyclonal | EPITOMICS                    |
| COX2 (sc-514489)   | 1:1000 |     |     | Mouse monoclonal | Santa Cruz Biotechnology     |
| c-Fos (sc-413)     | 1:1000 |     |     | Mouse monoclonal | Santa Cruz Biotechnology     |
| MDM2 (S160)        | 1:1000 |     |     | Rabbit polyclonal | Bioworld Technology         |
| VEGFA (sc-507)     | 1:1000 |     |     | Rabbit polyclonal | Santa Cruz Biotechnology     |