Supplementary Information for

MicroRNA-128-3p mediates lenvatinib resistance of hepatocellular carcinoma cells by downregulating c-Met

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Contents

Supplementary Materials and Methods

Name	Catalogue No.	Supplier	Size in WB
			(kDa)
Anti-cyclin D1 Ab	#2922	CST	36
Anti-Akt Ab	#4691	CST	60
Anti-phosphor-Akt (Ser473) Ab	#4060	CST	60
Anti-ERK Ab	#4695	CST	44, 42
Anti-p-ERK (Thr202/Tyr204) Ab	#4370	CST	44, 42
Anti-GSK-3β Ab	#12456	CST	46
Anti-p-GSK-3β (Ser9) Ab	#5558	CST	46
Anti-caspase-3 Ab	#9668	CST	35, 19
Anti-caspase-9 Ab	#9502	CST	47, 37
Anti-PARP	#9542	CST	116,89
Anti-β-actin Ab	sc-130065	Santa Cruz	45
FITC-conjugated rat anti-mouse	sc-516140	Santa Cruz	/
Ab			
Anti-Ki67 Ab	ab15580	Abcam	/
Anti-c-Met Ab	ab51067	Abcam	150
Anti- phosphor-c-Met	ab68141	Abcam	150
(Thr1349) Ab			
Recombinant human HGF	PHG0321	ThermoFisher	/
protein			
HRP-conjugated goat anti-	ab6789	Abcam	/
mouse			
Anti-Ki67 Ab	ab15580	Abcam	/
HRP goat anti-mouse Ab	TA130004	OriGene	/
HRP goat anti-rabbit Ab	TA140003	OriGene	/
HRP rabbit anti-goat Ab	TA130032	OriGene	/
HRP rabbit anti-rat Ab	TA130038	OriGene	/
VECTASTAIN [®] ELITE ABC kit	SK-4100	Vector	/
TUNEL kit	# 11684795910	Sigma-Aldrich	/
Lipofectamine2000	#11668019	Invitrogen	/
ССК-8	CK04-05	Dojindo	/
DAPI	d9564	Sigma-Aldrich	/
TRIzol™ Reagent	15596026	ThermoFisher	/
TaqMan [®] MicroRNA Reverse	4366597	Thermo Fisher	/
Transcription kit			

List of antibodies, main reagents and kits used in the study

TaqMan™	Reverse	N8080234	Thermo Fisher	/
Transcription Reagents				
SYBR Green		S7563	Thermo Fisher	
Lenvatinib		S5240	Selleckchem	/
Capmatinib		S2788	Selleckchem	/

Notes: Ab, antibody; CST, Cell Signaling Technology (Boston, MA, USA); DAPI, 4',6diamidino-2-phenylindole; Dojindo, Dojindo Molecular Technologies, Gaithersburg, MD, USA; FITC, fluorescein isothiocyanate; GSK-3β, glycogen synthase kinase 3β; HRP, horseradish peroxidase; Invitrogen (Carlsbad, CA, USA); R&D Systems (Minneapolis, MN, USA); OriGene (OriGene Technologies, Inc., Beijing, China); Santa Cruz; Santa Cruz Biotechnology (Santa Cruz, CA, USA); Sigma-Aldrich (St. Louis, MO, USA); S6K (ribosomal protein S6 kinase); TUNEL, Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling agent; Vector, Vector Laboratories (CA, USA); WB, Western blot analysis.

Cell proliferation assay

Cell proliferation was analyzed by using Cell Counting Kit-8 (CCK-8). Briefly, cells were seeded at 1×10^3 cells /well in 96-well plates. After treatments, the medium was replaced 100 µl of fresh medium containing 10 µl of CCK-8 reagent each well. Cells were incubated for 2 h at 37°C and the optical density (OD) at 450 nm was measured. Cell proliferation rate (%) was calculated by using a formula: (Experimental OD-Control OD)/Control OD × 100%.

Assessment of cell apoptosis in vitro

Cells were seeded at 5.0×10^5 cells/well in six-well plates and incubated with different reagents for 48 h, and then harvested and counted. A Cell Cycle kit (BD Biosciences,

Beijing, China) was used to determine the percentages of cells at different phases of cell cycle by using flow cytometry with a Beckman Coulter Epics Altra II cytometer (Beckman Coulter, California, USA). Cells (1×10^5) were incubated in 110µl of binding buffer containing 5µl of Annexin V and 5µl of PI for 15 min at room temperature in dark, and then subjected to flow cytometry to measure the apoptosis rate (%) with the cytometer. The stained cells were also visualized under laser scanning confocal microscopy.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Methods have been described in details previously [1-3]. Briefly, total RNA was extracted from cell lysates using TRIzol[®] reagent (cat. no. 15596026; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. RNA quantity and quality were measured using a NanoDrop ND-1000 system. For amplifying miRNAs, RNA was reversely transcribed into cDNA using a TaqMan[®] MicroRNA Reverse Transcription kit (cat no. 4366597; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and a stem-loop RT primer and pairs of primers (Table S1). U6 RNA expression served as a positive control. For amplifying mRNAs, RNA was reversely transcribed into cDNA using TaqMan[™] Reverse Transcription Reagents (cat. no. N8080234; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and pairs of primers (Table S1). The expression level of GAPDH served as an internal control. Following RT, qPCR was performed using an Applied Biosystems 7500 RealTime PCR system and SYBR Green (cat. no. S7563; Thermo Fisher Scientific, Inc.) according to the manufacturer's instruction. The thermocycling conditions were as follows: 10 min initial denaturation at 94°C, 15 sec denaturation at 94°C and 30 sec of annealing at 56°C (40 cycles) and final extension for 1 min at 72°C. Experiments were performed in triplicate, and the relative expression levels were calculated using the 2^{-} $\Delta\Delta Cq$ method [4].

Transfection of oligonucleotides in vitro

The overexpression or knockdown of miR-128-3p was achieved by transfecting cells with a miRNA mimic or inhibitor. The synthetic RNA molecules including miR-128-3p mimic, antagomiR-128-3p, scrambled mimics control and antagomiR control were purchased from GenePharma Co., Ltd. (Shanghai, China). Cells (1×10⁵/well) were seeded in 6-well plates and cultured with DMEM supplemented with 10% FBS. When cells were grown to 60-70% confluence, they were incubated with oligonucleotides at 50 nM using Lipofectamine[®] RNAiMAX transfection reagent (Thermo Fisher Scientific, Inc.) in serum-free media at 37°C for 48 h, and were then immediately subjected to subsequent assays.

Western blot analysis

Cells were homogenized in protein lysate buffer (50 mM Tris, pH 7.4, 100 μM EDTA, 0.25 M sucrose, 1% SDS, 1% NP40, 1 μg/ml leupeptin, 1 μg/ml pepstatin A and 100

µM phenyl methyl sulfonyl fluoride), and debris was removed via centrifugation at $10,000 \times g$ for 10 min at 4°C. Protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Inc.). Protein (30 µg/lane) were resolved on 10% SDS-polyacrylamide gels and then electrophoretically transferred to PVDF membranes, which were blocked in TBS-Tween 20 [137 mM NaCl, 20 mM Tris HCl (pH 7.6) and 0.1% (v/v) Tween 20] containing 5% (w/v) non-fat dry milk at 37°C for 2 h. The membranes were incubated at 4°C overnight with primary Abs, followed by an incubation with alkaline phosphatase-conjugated secondary Abs (1:2,000) for 2 h at room temperature in the dark. Membranes were then developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Tiangen Biotech Co., Ltd.). The density of each band was measured using the ChemiDoc XRS system (Version 3.0; Bio-Rad Laboratories, Inc.) and the semi-quantitative measurement of band density was repeated thrice. In preliminary experiments, serial dilutions of lysates (containing 2.5, 5, 10, 20, 40 or 80µg protein) were immunoblotted; band intensities were measured and plotted against protein amounts to generate a standard curve, and the amount of protein for each blot was determined.

Luciferase reporter assay

The full-length of 3'UTR of human c-Met mRNA (Gene ID: 4233) containing miR-128-3p-targeting sequence was inserted into an SV40 promoter-driving luciferase reporter vector (Ambion; Thermo Fisher Scientific, Inc.) to create SV40 promoterdriving luciferase reporter vector wild-type (WT) and mutated type (MT) pMIR-luc-MET-3'-UTR vectors (Figure 2A). Cells $(1 \times 10^4$ /well) were cultured in 24-well plates, and when at 60-70% confluence, they were co-transfected with the vectors (100 nM), together with 50 nM miR-128-3p mimics, 50 nM antagomiR-128-3p or 50 nM control oligonucleotides using Lipofectamine[®] 3000 (Thermo Fisher Scientific, Inc.) at 37°C. A luciferase reporter vector without the miR-128-3p targeting sequence was transfected in parallel. After transfection for 48 h, luciferase activities were measured using the Dual-luciferase Reporter Assay system (Promega Corporation) and normalized to Renilla according to the manufacturer's instructions. The relative luciferase activity in cells was expressed as a percentage of the luciferase activity over that of cells transfected with the vector without miR-128-3p targeting sequence.

Assessment of cell cycle

Cells were seeded at 5.0×10^5 cells/well in six-well plates and underwent different treatments for 48 h, and then harvested and counted. A Cell Cycle kit (BD Biosciences, Beijing, China) was used to determine the percentages of cells at different phases of cell cycle by using flow cytometry with a Beckman Coulter Epics Altra II cytometer (Beckman Coulter, California, USA).

Immunohistochemistry of assessing gene expression in tumor tissues

Cryosections (5 µm) of tumors harvested from animals were prepared, blocked with 3%

BSA, and incubated with primary Abs at 4°C overnight. They were subsequently incubated for 30 min with appropriate secondary Abs using the Ultra-Sensitive TMS-P kit (Zhongshan Co., Beijing, China), and immunoreactivity developed with Sigma FAST DAB (3,3'-diaminobenzidine tetrahydrochloride) and CoCl₂ enhancer tablets (Sigma-Aldrich, Shanghai, China). Sections were counterstained with hematoxylin, mounted, and examined by microscopy. Quantitative analysis of the expression of each gene was conducted by using ImageJ (Fiji Edition, National Institutes of Health, USA), and their expression was expressed as pixels/µm².

In situ Ki-67 proliferation index

Tumor sections were immunostained with an anti-Ki-67Ab as above and examined to count Ki-67 positive cells in 10 randomly selected \times 400 high-power fields under microscopy. The Ki-67 proliferation index was calculated according to the following formula: the number of Ki-67 positive cells/ the total cell count \times 100%.

In situ detection of apoptotic cells

The above tumor sections were stained with the TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) (Roche, Shanghai, China). The TUNEL positive cells were counted in 20 randomly selected \times 200 high-power fields under microscopy. The apoptosis index was calculated according to the following formula: the number of apoptotic cells \times /total number of nucleated cells \times 100%.

Supplementary Tables

Oligonucleotides		Sequences
Mimics	miR-1-3p	5'-UGGAAUGUAAAGAAGUAUGUAU-3'
	miR-19a	5'-UGUGCAAAUCUAUGCAAAACUGA-3'
	miR-20b-3p	5'-ACUGUAGUAUGGGCACUUCCAG-3'
	miR-23b-3p	5'-AUCACAUUGCCAGGGAUUACCAC-3'
	miR-27a-3p	5'-UUCACAGUGGCUAAGUUCCGC-3'
	miR-34a	5'-UGGCAGUGUCUUAGCUGGUUGU-3'
	miR-128-3p	5'-UCACAGUGAACCGGUCUCUUU-3'
	miR-144-3p	5'-UACAGUAUAGAUGAUGUACU-3'
	miR-182	5'-UUUGGCAAUGGUAGAACUCACACU-3'
	miR-206	5'-UGGAAUGUAAGGAAGUGUGUGG-3'
	miR-454-3p	5'-UAGUGCAAUAUUGCUUAUAGGGU-3'
Antagomil	R-128-3p	5'-AAAGAGACCGGUUCACUGUGA-3'
Mimics ne	gative control	5'-CAGUACUUUUGUGUAGUACAA-3'
Antagomil	R negative	5'-UCACAACCUCCUAGAAAGAGUAGA-3'
control		

Table S1. Sequences of miRNA mimics, antagomiR-128-3p and negative control oligonucleotides

Table S2. Primers for microRNAs and mRNAs

Genes	Primers	Sequences
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCATACATTA-3'
	Forward	5'-GGG CGC GTG GAA TGT AAA GAA G-3'
miR-1-3p	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCTCAGTTTTG-3'
	Forward	5'-GGC CGG TGT GCA AAT CTA TGC-3'
miR-19a	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCTGGAAG-3'
miR-20b-3p	Forward	5'-CGA CGC ACT GTA Antagomir TGG GCA C-3'

	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCGTGGTA-3'
	Forward	5'-GTG GCA TCA CAT TGC CAG GG-3'
miR-23b-3p	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GA TACG AC -3'
	Forward	5'-GGC TGG CAG TGT CTT AGC TG -3'
miR-34a	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCGCGGAAC-3'
	Forward	5'-GCA GGC TTC ACA GTG GCT AAG -3'
miR-27a-3p	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCAAAGAG-3'
	Forward	5'-GCG TGT TCA CAG TGA ACC GGT C-3'
miR-128-3p	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCAGTACATC-3'
	Forward	5'-GGC GGC GCT ACA GTA TAG ATG ATG -3'
miR-144-3p	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CCAGTGTGAG-3'
	Forward	5'-GCC GAC GTT TGG CAA TGG TAG -3'
miR-182	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	T	1
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCACACAC-3'
	Forward	5'-GG CTG GAA TGT AAG GTG TGT GG-3'
miR-206	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	T	1
	RT primer	5'-GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GATACG ACA CAA CCACCCTATA-3'
	Forward	5'-GCC GGC GTA GTG CAA TAT TGC -3'
miR-454-3p	Reverse	5'-CAG TGC AGG GTC CGA GGT AT-3'
	Forward	5'-CTCGCTTCGGCAGCACA-3'
U6 RNA	Reverse	5'-AACGCTTCACGAATTTGCGT-3'
	Forward	5'-GAAATCCCATCACCATCTTCCAGG-3'
GAPDH	Reverse	5'-GAGCCCCAGCCTTCTCCATG-3'
	Forward	5'-CTAGACACATTTCAATTGGT-3'
c-Met	Reverse	5'-TGTTGCAGGGAAGGAGTGGT-3'

	Forward	5'-ACCATGTGGGTGACCAAACT-3'
HGF	Reverse	5'-TGTGTTCGTGTGGTATCATGG-3'

Notes: RT, reverse transcription; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Supplementary Figures

Figure S1



Figure S1 Animal experimental schedules. Huh7 or Huh7-LR cells (5×10^6) were subcutaneously injected into the flake of mice. When Huh7 or Huh7-LR tumors grew to ~100 mm³ in volume 15 or 20 days after cell inoculation, respectively, the mice were randomly assigned to treatment groups. In the model of Huh7-LR tumors, the mice received an oral administration of lenvatinib (2 mg/kg) daily after they were injected with Huh7-LR cells until they were assigned to treatment groups.





Figure S2 Lenvatinib-resistant HCC cells are refractory to lenvatinib-induced proliferation inhibition and apoptosis. (A) The lenvatinib-resistant cells, SMMC-LR and Huh7-LR, and their respective parentals, SMMC-7721 and Huh7 cells, were incubated with lenvatinib at serials of concentrations for 48 h. Cell proliferation (%) was detected and normalized to mock-treated cells. (B-D) The above cells were incubated with 0, 0.5 or 1.5 μ M of lenvatinib for 48 h, and then subjected to flow cytometrical analysis to detect apoptosis (B) and the rates of apoptosis were determined (C). (D) Cell lysates were subjected to Western blot analysis, and band densities were normalized to β -actin. "*" P<0.05; "**P<0.001".





Figure S3 Capmatinib enhances the therapeutic effect of lenvatinib against Huh7-LR tumors in mice. (A) Animal experimental schedules. (B) Huh7 or Huh7-LR tumors were established in mice and when they grew to ~100 mm³, the mice were assigned to different groups and received daily oral administration of vehicle or 10 mg/kg lenvatinib for 15 days. (C) Huh7-LR tumors were established and when they grew to ~100 mm³, the mice were assigned to four groups, which received daily administration of vehicle, lenvatinib (10 mg/kg) or capmatinib (30 mg/kg) or the combination for 15 days. Tumor volumes were measured every 3 days and harvested at the end of experiments. "*" P<0.05; "**P<0.001". The values of coefficient of drug interaction (CDI) were calculated according to the following formula: CDI =AB/A×B, where AB

is the ratio of the combination group to the control group (vehicle), while A or B is the ratio of each drug group to the control group; "CDI < 0.7" indicates a synergistic effect, " $0.7 \le$ CDI <1", an additive effect, and "CDI \ge 1", an antagonistic effect [3, 5].

Figure S4



Figure S4 Inhibition of c-Met by capmatinib enhances the sensitivity of lenvatinib-resistant HCC cells to lenvatinib. (A) Huh7, Huh7-LR, SMMC-7721 and SMMC-LR cells were cultured for 48 h with various concentrations of capmatinib and subjected to cell proliferation assays. The values of half-maximal inhibitory concentration (IC50) were calculated. (B) Huh7-LR and SMMC-LR cells

were incubated for 48 h with capmatinib at various concentrations (0, 2, 4, 8 or 16 nM) in the presence or absence of lenvatinib (0.5 μ mol/L), and then subjected to cell proliferation assays. The values of coefficient of drug interaction (CDI) were calculated as described in Figure S3. (C) Huh7-LR and SMMC-LR cells were incubated for 48 h with lenvatinib (0.5 μ mol/L), capmatinib (2 nM) or the combination, and subjected to cytometry for measuring cell apoptosis (%). "**P<0.001".

Figure S5

Position 499-505 of MET 3' UT	R 5'.	UCACCCAUUAGGUAAACAUUCCC	Position 1563-1570 of M	ET 3' UTR 5'	GCCACAAAAACACUGCACUGUGA
hsa-miR-1-3p	3'	UAUGUAUGAAGAAAUGUAAGGU	hsa-miR-128-3p	3'	UUUCUCUGGCCAAGUGACACU
Position 1308-1315 of MET 3'	JTR 5'	UGUGUUUUAUGUUAAGCAAAACA			
hsa-miR-19a-5p	3'	ACAUCACGUUGAUACGUUUUGA	Position 1430-1436 of M	MET 3' UTR 5	·GAAUUUUGUGCUUGCUACUGUAU
Position 631-637 of MET 3' U	TR 5'.	UCCCGAAUAGCUGGGACUACAGG	115d-11111-144-5p	,	UCAUGUAGUAGAUAUGACAU
hsa-miR-20b-3p	3'	GACCUUCACGGGUAUGAUGUCA			
			Position 173-179 of MET	3' UTR 5'	AUCUGACAGAGCAUCAGAACCAG
Position 2065-2072 of MET 3' U	TR 5'	UCUUGAGAACACUGCAAUGUGAA	hsa-miR-182-3p	3'	AUCAACCGUUCAGAUCUUGGU
hsa-miR-23b-3p	3'	CCAUUAGGGACCGUUACACUA			
			Position 814-820 of ME	T 3' UTR 5'	UAAAUUUUUGUAUAGACAUUCCU
Position 51-57 of MET 3' UTR	5'	GUCCAAUGGUUUUUUCACUGCCU	hsa-miR-206	3'	GGUGUGUGAAGGAAUGUAAGGU
hsa-miR-34a-5p	3'	UGUUGGUCGAUUCUGUGACGGU			
			Position 100-107 of ME	ET 3' UTR 5'	
Position 1564-1571 of MET 3' U	JTR 5'	CCACAAAAACACUGCACUGUGAA			111111
hsa-miR-27a-3p	3'	CGCCUUGAAUCGGUGACACUU	hsa-miR-454-3p	3'	UGGGAUAUUCGUUAUAACGUGAU

Figure S5 Potential miRNAs that have well-conserved binding sites of 3'-UTR of human MET gene. Eleven potential miRNAs that have putative binding sites with the 3'UTR of human c-Met gene are screened out by using multiple miRNA prediction tools.

Figure S6



Figure S6 MiR-128-3p inhibits cell cycle progression at the G0/1 phase of lenvatinib-resistant HCC cells. Huh7-LR cells were transfected for 48 h with negative control (NC) or miR-128-3p mimics oligonucleotides, followed by incubation in the presence or absence of lenvatinib (0.5μ mol/L) for 24 h, and then subjected to flow cytometry for detecting cell cycle distribution (A) and percentages of cells at different phases were plotted (B). The percentages of cells arrested at the G0/1 phase were compared by using a one-way ANOVA with a Tukey post-hoc test. "*" P<0.05; "**P<0.001".

Figure S7



Figure S7 MiR-128-3p enhances lenvatinib-induced apoptosis of HCC cells. Huh7 and Huh7-LR cells were transfected for 48 h with negative control (NC), miR-128-3p mimics or antagomiR-128-3p oligonucleotides, and then incubated in the presence or absence of lenvatinib (0.5μ mol/L) for 24 h. (A) Representative dot plots are taken from flow cytometric analysis for measuring cell apoptosis (%) in Figure 6B. (B) Representative images were taken from cells stained with Annexin V/propidium iodide (magnification × 100).

Figure S8



Figure S8 Gene delivery of miR-128-3p mimics inhibits the activation of c-Met pathway in Huh7-LR tumors. (A) Illustrated are representative tumor sections immunostained with specific Abs as indicated. These tumors were harvested in Figure 7. Magnification bar = 100 μ m. (B) The expression of each protein was expressed as pixels/ μ m² by using ImageJ software. "*" P<0.05; "**P<0.001".

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Uncropped Western blot images



Fig.1B Anti-p-Met

Fig.1B Anti-c-Met































Fig.3D Huh7-Anti-actin



Fig.3D SMMC-Anti-c-Met



Fig.3D SMMC-Anti-actin



Fig.4EF Anti-c-Met



Fig.4EF Anti-ERK



Fig.4EF Anti-p-ERK



Fig.4EF Anti-cyclin D1



Fig.4EF Anti-actin



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Fig.5CD Anti-c-Met
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Fig.5CD Anti-Akt



Fig.5CD Anti-p-Akt







Fig.5CD Anti-p-GSK



Fig.5CD Anti-caspase-9







Fig.5CD Anti-actin



Fig.S2D Huh7-Anti-cas-3







Fig.S2D Huh7-Anti-atin



Fig.S2D SMMC-Anti-cas-3



Fig.S2D SMMC-Anti-PARP



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Fig.S2D SMMC-Anti-actin



Densitometric analysis of Western blot bands

Figure 1 B		Densitor	Densitometric readings			Ratio to actin			
c-Met	Huh7	12658	9165	17652	0.2020	0.1463	0.2817		
	Huh7-LR	83632	93354	76787	1.3347	1.4898	1.2255		
	SMMC-7721	18864	21931	24998	0.3011	0.3500	0.3989		
	SMMC-LR	52864	68941	60535	0.8437	1.1002	0.9661		
p-									
Met	Huh7	8032	12039	4366	0.1282	0.1921	0.0697		
	Huh7-LR	88145	69236	81353	1.4067	1.1049	1.2983		
	SMMC-7721	11653	8287	15776	0.1860	0.1323	0.2518		
	SMMC-LR	55742	59124	52436	0.8896	0.9436	0.8368		
beta-ad	ctin								
	Huh7	61638	79488	53348					
	Huh7-LR	60498	63362	49565					
	SMMC-7721	69002	57416	64310					
	SMMC-LR	56178	62059	75056					
Figure 1C		Densitor	netric read	linas	Ratio to a	actin			
.									
	Huh7								
c-Met	(0)	24035	15896	19952	0.2649	0.1752	0.2199		
	Huh7 (1.5)	56653	39131	46757	0.6244	0.4313	0.5153		

	Huh7-LR (0)	109590	115875	103892	1.2078	1.2771	1.1450
	Huh7-LR (1.5)	150820	138951	143020	1.6623	1.5314	1.5763
	SMMC-7721 (0) SMMC-7721	34051	23963	26366	0.3753	0.2641	0.2906
	(1.5)	37421	56023	45375	0.4124	0.6175	0.5001
	SMMC-LR (0)	65208	48399	55154	0.7187	0.5334	0.6079
	SMMC-LR (1.5)	99241	88561	92559	1.0938	0.9761	1.0201
	Huh7						
p-Met	(0)	18023	25825	21479	0.1986	0.2846	0.2367
	Huh7 (1.5)	63461	49214	53364	0.6994	0.5424	0.5881
	Huh7-LR (0)	76224	65821	64823	0.8401	0.7254	0.7144
	Huh7-LR (1.5)	139792	130265	130071	1.5407	1.4357	1.4336
	SMMC-7721 (0) SMMC-7721	21315	28863	28758	0.2349	0.3181	0.3170
	(1.5)	49568	35015	43349	0.5463	0.3859	0.4778
	SMMC-LR (0)	69523	54841	60729	0.7662	0.6044	0.6693
	SMMC-LR (1.5)	74198	88714	84785	0.8178	0.9778	0.9345
	Huh7						
Akt	(0)	116989	93754	96838	1.2894	1.0333	1.0673
	Huh7 (1.5)	94687	112685	95613	1.0436	1.2420	1.0538
	Huh7-LR (0)	113685	109856	105816	1.2530	1.2108	1.1662
	Huh7-LR (1.5)	104580	97658	97177	1.1526	1.0763	1.0710
	SMMC-7721 (0) SMMC-7721	84715	74141	77954	0.9337	0.8171	0.8592
	(1.5)	78369	90217	73669	0.8637	0.9943	0.8119
	SMMC-LR (0)	78465	89219	82636	0.8648	0.9833	0.9108
	SMMC-LR (1.5)	69235	83122	79009	0.7631	0.9161	0.8708
	Huh7						
p-Akt	(0)	19995	11725	14653	0.2204	0.1292	0.1615
	Huh7 (1.5)	47116	38081	42835	0.5193	0.4197	0.4721
	Huh7-LR (0)	67965	80301	74934	0.7491	0.8850	0.8259
	Huh7-LR (1.5)	120268	112470	104785	1.3255	1.2396	1.1549
	SMMC-7721 (0) SMMC-7721	24990	20341	22718	0.2754	0.2242	0.2504
	(1.5)	43025	36892	39849	0.4742	0.4066	0.4392
	SMMC-LR (0)	52892	36657	43827	0.5829	0.4040	0.4830
	SMMC-LR (1.5)	84352	75145	80035	0.9297	0.8282	0.8821

beta-	Huh7			
actin	(0)	80553	87083	81480
	Huh7 (1.5)	91883	96806	62085
	Huh7-LR (0)	78871	99822	82448
	Huh7-LR (1.5)	94280	106073	87607
	SMMC-7721 (0)	101607	96835	93233
	SMMC-7721			
	(1.5)	93812	89103	72225
	SMMC-LR (0)	112043	115151	100079
	SMMC-LR (1.5)	84523	88649	81320

Figure 3D	Densitometric readings			Ratio to actin			
c-Met	Huh7 (mock)	30642	26395	29454		0.3295	0.2838
	Huh7 (NC)	23684	31954	25274		0.2547	0.3436
	Huh7 (anta)	46236	59261	53537		0.4972	0.6372
	Huh7-LR (mock)	80626	87561	88498		0.8669	0.9415
	Huh7-LR (NC)	84728	80017	83570		0.9110	0.8604
	Huh7-LR (mimics)	28061	17578	24113		0.3017	0.1890
beta-actin							
	Huh7 (mock)		87984	97275			
	Huh7 (NC)		103225	89851			
	Huh7 (anta)		101185	91184			
	Huh7-LR (mock)		84054	85663			
	Huh7-LR (NC)		92192	111665			
	Huh7-LR (mimics)		73076	110059			
c-Met							
	SMMC						
	(mock) 27014 SMMC	18184	23488		0.3158	0.2126	0.2746
	(NC) 24698	17645	19244		0.2887	0.2063	0.2250
	SMMC (anta)	45658	37410	40106		0.5338	0.4373
	SMMC-LR (mock)	82584	64123	76547		0.9655	0.7496
	SMMC-LR (NC)	69510	83451	77992		0.8126	0.9756
	SMMC-LR (mimics)	22147	29651	25186		0.2589	0.3466
beta-actin	SMMC (mock)		71960	102075			
	SMMC						
	(NC)	77444	87524				
	SMMC (anta)		81246	61379			
	SMMC-LR (mock)		102568	102514			

SMMC-LR (NC)	79255	83347
SMMC-LR (mimics)	80662	87192

Figure 4E		Densitome	Densitometric readings			Ratio to actin		
Huh7 c-Met								
	NC	25141	18252	21232	0.2920	0.2120	0.2466	
	Lenvatinib	26654	36541	29794	0.3096	0.4244	0.3460	
	AntagomiR	53961	47512	47160	0.6267	0.5518	0.5477	
	Lenvatinib +							
	AntagomiR	65952	56741	52953	0.7660	0.6590	0.6150	
ERK								
	NC	87410	78369	89941	1.0152	0.9102	1.0446	
	Lenvatinib	100451	85654	92862	1.1667	0.9948	1.0785	
	AntagomiR	95052	89234	106047	1.1040	1.0364	1.2317	
	Lenvatinib +							
	AntagomiR	91234	86541	88277	1.0596	1.0051	1.0253	
p-ERK								
	NC	46682	35410	39310	0.5422	0.4113	0.4566	
	Lenvatinib	17022	9728	14499	0.1977	0.1130	0.1684	
	AntagomiR	79210	68320	64278	0.9200	0.7935	0.7465	
	Lenvatinib +							
	AntagomiR	32628	24741	27871	0.3790	0.2873	0.3237	
cyclin D1								
o) o	NC	63630	51201	58232	0.7390	0.5947	0.6763	
	Lenvatinib	21639	31741	26694	0.2513	0.3686	0.3100	
	AntagomiR	84692	73821	71376	0.9836	0.8574	0.8290	
	Lenvatinib +							
	AntagomiR	49923	39105	45290	0.5798	0.4542	0.5260	
beta-actin								
	NC	77546	90558	92955				
	Lenvatinib	95510	91908	82487				
	AntagomiR	85758	80155	90558				

Lenvatinib +			
AntagomiR	78497	81123	86165

Figure 4F	igure 4F De		Densitometric readings			Ratio to actin		
Huh7-LR c-Met								
	NC	78714	87629	89337	0.9328	1.0385	1.0587	
	Lenvatinib	116691	98241	99141	1.3829	1.1642	1.1749	
	Mimics	51821	36369	45978	0.6141	0.4310	0.5449	
	Lenvatinib +							
	Mimics	83269	71741	88013	0.9868	0.8502	1.0430	
FRK								
	NC	75365	90124	82597	0.8931	1.0680	0.9788	
	Lenvatinib	68146	82652	74504	0.8076	0.9795	0.8829	
	Mimics	79638	68610	84649	0.9438	0.8131	1.0032	
	Lenvatinib +							
	Mimics	92632	78500	87079	1.0978	0.9303	1.0319	
p-ERK								
	NC	78692	88143	93908	0.9326	1.0446	1.1129	
	Lenvatinib	70252	60521	59088	0.8325	0.7172	0.7002	
	Mimics	52362	40621	43717	0.6205	0.4814	0.5181	
	Lenvatinib +							
	Mimics	34692	26695	29746	0.4111	0.3164	0.3525	
cyclin D1								
-	NC	72359	53864	61107	0.8575	0.6383	0.7242	
	Lenvatinib	51643	57910	52462	0.6120	0.6863	0.6217	
	Mimics	36621	46265	38625	0.4340	0.5483	0.4577	
	Lenvatinib +							
	Mimics	25114	20365	20339	0.2976	0.2413	0.2410	
heta_actin								
	NC	71752	101867	94726				
	Lenvatinih	77226	87316	71599				
	Mimics	R1U38	8/201	90305				
	Lenvatinih +	01000	04001	20202				
	Mimics	85855	86103	79998				

Figure 5C Huh7			Densitometric readings			Ratio to actin		
c-Met								
	NC		14998	18992	17469	0.2186	0.2768	0.2546
	Lenvatinib		27410	20914	25777	0.3995	0.3048	0.3757
	AntagomiR		36892	30100	35926	0.5377	0.4387	0.5236
	Lenvatinib + Antagor	niR	51921	41320	46727	0.7567	0.6022	0.6810
p-Akt								
	NC		15465	8741	12844	0.2254	0.1274	0.1872
	Lenvatinib		25140	34624	32862	0.3664	0.5046	0.4790
	AntagomiR		40101	28245	36630	0.5845	0.4117	0.5339
	Lenvatinib + Antagor	niR	63351	52264	55229	0.9233	0.7617	0.8050
p-GSK3k	oeta							
	NC		9410	19523	14293	0.1372	0.2845	0.2083
	Lenvatinib		33470	25419	29620	0.4878	0.3705	0.4317
	AntagomiR		45130	37852	34344	0.6578	0.5517	0.5006
	Lenvatinib + Antagor	niR	57942	51008	53660	0.8445	0.7434	0.7821
cleaved	caspase-9							
	NC		3185	1874	1116	0.0464	0.0273	0.0163
	Lenvatinib		12796	10520	10676	0.1865	0.1533	0.1556
	AntagomiR		1695	236	127	0.0247	0.0034	0.0019
	Lenvatinib + Antagor	niR	7745	5730	9167	0.1129	0.0835	0.1336
cleaved	caspase-3							
	NC		3192	2382	2659	0.0465	0.0347	0.0388
	Lenvatinib		14068	10848	12134	0.2050	0.1581	0.1769
	AntagomiR		451	1987	2457	0.0066	0.0290	0.0358
	Lenvatinib + Antagor	niR	8864	5557	6262	0.1292	0.0810	0.0913
actin								
	NC	71845	79695	68800				
	Lenvatinib	64852	81893	6179				
	AntagomiR	64517	79078	82655				

	Lenvatinib + AntagomiR	71049	67673	85107	
Figure 5D		Densitometric readings			Ratio to actin
Huh7-LR					
c-Met					

c-Met							
	NC	98652	88412	87114	1.0902	0.9771	0.9627
	Lenvatinib	103878	127624	112398	1.1480	1.4104	1.2421
	Mimics	54523	40122	49231	0.6025	0.4434	0.5441
	Lenvatinib +						
	Mimics	97552	76210	86843	1.0781	0.8422	0.9597
p-Akt		76641	67507	70200	0.0470	07460	0 7760
	NC	70041 02614	60241	70289	0.8470	0.7403	0.7708
	Lenvalinio	03014	10010	11890	0.9240	0.7052	0.0000
	Iviimics	31903	18010	23322	0.3532	0.1990	0.2577
	Mimics	46555	30149	37311	0.5145	0.3332	0.4123
n CSK3ho	ta						
p-030306	NC	89677	74555	82801	0 9910	0.8239	0 9150
	Lenvatinib	96358	109790	96977	1 0649	1 2133	1 0717
	Mimics	47628	34941	39589	0.5263	0.3861	0.4375
	Lenvatinib +						
	Mimics	70951	62402	67530	0.7841	0.6896	0.7463
cleaved ca	onace_0						
		3101	1623	605	0 0353	0 0179	0 0067
	Lenvatinih	13369	7874	9518	0.0333	0.0175	0.0007
	Mimics	22653	11682	17243	0.2503	0.1291	0.1002
	Lenvatinib +	22000	11002	11210	0.2000	0.1201	0.1000
	Mimics	31022	26840	26292	0.3428	0.2966	0.2906
cleaved ca	spase-3						
2.00100.00	NC	3666	2010	2468	0.0405	0.0222	0.0273
	Lenvatinib	15791	10205	12010	0.1745	0.1128	0.1327
	Mimics	27803	16647	23416	0.3073	0.1840	0.2588
	Lenvatinib +						
	Mimics	42120	33294	35886	0.4655	0.3679	0.3966

beta-ad	ctin						
	NC	92426	100372	79113			
	Lenvatinib	93521	83205	96894			
	Mimics	84867	90371	85921			
	Lenvatinib +						
	Mimics	96010	105118	78046			
Figure	S2D	Densitomet	ric readinc	IS	Ratio to a	ctin	
caspase	2-3		-				
	Huh7 (0)	1099	.31	151	0.0135	0 0004	0.0019
	Huh7 (0.5)	32658	24641	27879	0.4005	0.3022	0.3419
	Huh7 (2)	46621	38745	34508	0.5717	0.4751	0.4232
	Huh7-LR (0)	108	1668	49	0.0013	0.0205	0.0006
	Huh7-LR (0.5)	2913	912	1407	0.0357	0.0112	0.0173
	Huh7-LR (2)	9356	15043	7404	0.1147	0.1845	0.0908
PARP							
	Huh7 (0)	11325	8102	7484	0.1389	0.0994	0.0918
	Huh7 (0.5)	17913	12798	15771	0.2197	0.1569	0.1934
	Huh7 (2)	37217	30452	32634	0.4564	0.3734	0.4002
	Huh7-LR (0)	154	1524	954	0.0019	0.0187	0.0117
	Huh7-LR (0.5)	1624	545	317	0.0199	0.0067	0.0039
	Huh7-LR (2)	4042	6795	8534	0.0496	0.0833	0.1047
beta-ac	tin						
	Huh7 (0)	77951	86973	86264			
	Huh7 (0.5)	83409	83043	77652			
	Huh7 (2)	75281	87174	69173			
	Huh7-LR (0)	80578	79651	86181			
	Huh7-LR (0.5)	90460	72065	86048			
	Huh7-LR (2)	83923	70949	91064			
636D366	<u>, 2</u>						
caspast	SMMC-7721 (0)	57	856	207	0 0006	0 0097	0 0023
	SMMC-7721 (0)	27871	17865	23195	0.3154	0.2022	0.2625
	SMMC-7721 (2.0)	40326	48620	48917	0.0104 0.4563	0.55022	0.5525
	SMMC-IR (0)	114	921	25	0.0013	0.0104	0.0003
	SMMC-LR (0.5)	2674	1834	3445	0.0303	0.0208	0.0390
	SMMC-LR (2.0)	11939	5752	8821	0.1351	0.0651	0.0998
			0.02		0.2002		2.3000

	SMMC-7721 (0)	42	1163	524	0.0005	0.0132	0.0059
	SMMC-7721 (0.5)	12307	17692	12420	0.1393	0.2002	0.1405
	SMMC-7721 (2.0)	46052	39852	38703	0.5211	0.4509	0.4379
	SMMC-LR (0)	250	877	104	0.0028	0.0099	0.0012
	SMMC-LR (0.5)	1835	25	464	0.0208	0.0003	0.0053
	SMMC-LR (2.0)	8960	12382	10472	0.1014	0.1401	0.1185
actin							
	SMMC-7721 (0)	73471	81233	71366			
	SMMC-7721 (0.5)	87594	89090	72212			
	SMMC-7721 (2.0)	79442	87477	78858			
	SMMC-LR (0)	92030	95138	100066			
	SMMC-LR (0.5)	101594	96822	93220			
	SMMC-LR (2.0)	94267	106060	90799			

The End