Additional File 1

Exosomal miR-6126 as a novel therapeutic target for overcoming resistance of anti-cancer effect in hepatocellular carcinoma

Hyemin Hwang^{1,#}, Jimin Kim^{1,#}, Tae-Hun Kim², Yeonju Han¹, Dayoung Choi¹, Sua Cho¹, Seunghwan Kim¹, Sanghee Park¹, Taehyun Park¹, Filippo Piccinini^{3,4}, Won Jong Rhee⁵, Jae-Chul Pyun², Misu Lee^{1,6,*}

¹Division of Life Sciences, College of Life Science and Bioengineering, Incheon National University, Incheon, 22012, Republic of Korea

²Department of Materials Science and Engineering, Yonsei University, 50 Yonsei-Ro, Seodaemun-Gu, Seoul, 03722, Republic of Korea.

³ IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy

⁴ Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy

⁵ Department of Bioengineering and Nano-Bioengineering, Incheon National University Incheon 22012, Republic of Korea.

⁶Institute for New Drug Development, College of Life Science and Bioengineering, Incheon National University, Incheon, 22012, Republic of Korea

[#] These authors contributed equally to this study.

Corresponding author: Misu Lee, PhD

Incheon National University, Incheon, Republic of Korea

E-mail: misulee@inu.ac.kr; Tel.: +82 32 835 8091; Fax: +82 32 835 0754

Antibodies	Company	Cat. No.	Dilution
pERK1/2	CST	4370	1:1000
LDHA	CST	3582T	1:1000
HK2	Abcam	ab209847	1:500
CD44	Abcam	ab157107	1:1000
TSG101	Abcam	ab275018	1:500
CD63	SBI	EXOAB-CD63A-1	1:1000
Calnexin	Abcam	ab275018	1:1000
β-actin	Santacruz	sc-477778HRP	1:5000

Table S1. Summary of antibodies.



Fig S1. Cell proliferation of control Huh7 cells (Huh7) and Huh7 resistant cells (Huh7R) after treatment with indicated concentrations of sorafenib for 48 hours. Data shown are the mean of three independent experiments \pm SD.



Fig S2. Densitometry intensity ratio of Fig 1A from replicated WB (n=3). *; p < 0.05; **; p < 0.01.



Fig S3. Expression levels of miR-4516, miR-6126, and miR-148a-3p from Huh7 and Huh7R cells. (A-C) Huh7 and Huh7R cells were treated with 20 μ M of sorafenib for indicated time. Total RNA of cell lysate was used to determine the expression levels of miR-4516 (A), miR-6126 (B), and miR-148a-3p (C). The results were normalized to rRNA using the 2– $\Delta\Delta$ Ct method. (**P < 0.01; ***P < 0.001; ****P < 0.0001)



Fig S4. (A) Huh7R cells were transfected with 500 pM negative control or miR-6126 mimic for 72 h. Cells were treated with 20 µM sorafenib (SOR) for 48 h and proteins were extracted. The expression levels of p-ERK1/2, ERK 1/2, HK2, and β -actin were analyzed by western blotting. (B) Viability of SK-Hep1R cells and (C) expression levels of p-ERK1/2, ERK 1/2, HK2, CD44 and β -actin analyzed using western blotting. SK-Hep1R cells were transfected with negative control (NC) or miR-6126 mimic and then treated with 20 µM sorafenib (SOR) for 48 h for viability assay and protein extraction. (D) Huh7 cells were transfected with 100 nM negative control or miR-6126 inhibitors for 72 h. Cells were treated with 20 µM sorafenib (SOR) for 48 h and proteins were extracted. The expression levels of p-ERK 1/2, ERK 1/2, HK2, CD44, and β -actin were analyzed by western blotting.



Figure S5. Densitometry intensity ratio of Fig 5C from replicated WB (n=3). **; p < 0.01, ***; p < 0.001.