## EGFR inhibition reverses resistance to lenvatinib in hepatocellular carcinoma cells

## **Supporting information**

Figure S1. IC50 of Hep3B, LR7.5-3B and HepG2 by lenvatinib treatment.

In vitro 24 h anti-proliferative activity of lenvatinib in three human HCC cell lines,

Hep3B, HepG2 and lenvatinib-resistant (LR)cells. The IC50 of Hep3B and LR cells were

2.8µM and>30µM, respectively. We also analyzed HepG2 cells, but IC50 was>39µM.

Figure S2-5.

Western blot analysis of the complete blot image, together with the recording of the

calculation of the p-protein/total protein ratio in a semi-quantitative manner.

Figure S6.

The inhibition of EGFR or IGF1R by erlotinib or linstinib treatment.

Figure S7.

Putative mechanism for lenvatinib resistance in Hep3B cells.

**Figure S1.**Determination of inhibitory concentrations at 50% (IC50) values for Lenvatinib in HCC cell lines incubated for 24 hours.



In vitro 24 h anti-proliferative activity of lenvatinib in three human HCC cell lines, Hep3B, HepG2 and lenvatinib-resistant (LR)cells. The IC50 of Hep3B and LR cells were 2.8µM and>30µM,respectively. We also analyzed HepG2 cells, but IC50 was>39µM.

## Figure S2-5. Full blot images of Western blotting assay



Figure1(A) Hep3B Semi-quantification of p-ERK/ERK was performed using Image J

Figure1(B) Huh7 Semi-quantification of p-ERK/ACTB was performed using Image J



Figure1(C) JHH-4 Semi-quantification of p-ERK/TUBB was performed using Image J







Figure1(C) Huh6 Semi-quantification of p-ERK/TUBB was performed using Image J



Figure1(D) HepG2 Semi-quantification of p-ERK/TUBB was performed using Image J



Figure2(A) Hep3B and LR7.5-3B Semi-quantification of p-ERK/ACTB was performed using Image J







Figure2(B) JHH-4(7.5) Semi-quantification of p-ERK/TUBB was performed using Image J







Figure2(B) Huh6(1.875) Semi-quantification of p-ERK/TUBB was performed using Image J



Figure3(E) 3B and LR Semi-quantification of p-EGFR/ACTB was performed using Image J



Figure3(F) 3B and LR Semi-quantification of p-INSR/ACTB was performed using Image J





Figure4(A) Hep3B and LR7.5-3B Semi-quantification of p-ERK/ACTB was performed using Image J







Figure4(D) Hep3B and LR7.5-3B Semi-quantification of p-ERK/ACTB was performed using Image J







Figure5(A) Hep3B and LR7.5-3B Semi-quantification of p-ERK/ACTB was performed using Image J



Figure5(D) Hep3B and LR7.5-3B Semi-quantification of p-ERK/ACTB was performed using Image I



Figur6(C) Hep3B and LR7.5-3B Semi-quantification of p-ERK/ACTB and p-EGFR/ACTB



ACTB:43kDa

EGFR:175kDa

Figure S6. The inhibition of EGFR or IGF1R by erlotinib or linstinib treatment,



