## Supplemental Materials: Downregulation of SHIP2 by Hepatitis B Virus X Promotes the Metastasis and Chemoresistance of Hepatocellular Carcinoma through SKP2

Kuo-Jung Su and Yung-Luen Yu



**Figure S1.** Expression of SHIP2 in human HCC tissue without or with HBV infection was examined by IHC analysis of the TMA, which was stained with human SHIP2 antibody.



**Figure S2.** SHIP2 is polyubiquitinated by the E3 ubiquitin ligase SKP2 in HEK293 cells. HEK293 cells were transfected with SKP2 expression vector for 48h. A except that cells were pretreated with bortezomib (50 nM) for 16h prior to cell harvest. Whole protein lysates were prepared followed by immunoprecipitation (IP) with anti-SHIP2. The precipitate was then electrophoresed on a polyacrylamide gel followed by immunoblot (IB) detection using anti-ubiquitin and anti-SKP2.







Paclitaxel





**Figure S3.** Knockdown of SHIP2 did not induce resistance to Iressa, Tarceva, Doxorubicin, Sorafenib, and Paclitaxel in Hep3B cells. The cytotoxic effects of Iressa, Tarceva, Doxorubicin, Sorafenib, and Paclitaxel in Hep3B cells. The Hep3B cells were plated in 96-well plate after SHIP2 knockdown for 72 h. The cells were treated with indicated doses (12.5  $\mu$ M and 25  $\mu$ M) of chemotherapy drugs for 48 h.



S5 of S11





**(B)** 



**Figure S4.** Raw data of Western blots from Figure 2. (**A**) Raw data from Figure 2A. (**B**) Raw data from Figure 2B. (**C**) Raw data from Figure 2C.







**(B)** 





**Figure S5.** Raw data of Western blots from Figure 3. (**A**) Raw data from Figure 3A. (**B**) Raw data from Figure 3B. (**C**) Raw data from Figure 3C.





**Figure S6.** Raw data of Western blots from Figure 5. (**A**) Raw data from Figure 5A. (**B**) Raw data from Figure 5C.





Figure S7. Raw data of Western blots from Figure S2.

| Table S1 | Used | in | This | Study | ζ. |
|----------|------|----|------|-------|----|
|----------|------|----|------|-------|----|

| Target Name |   | Sequence                               |
|-------------|---|--|
| E-cadherin  | F | 5'-GGACCAGGACTTTGACTTGAGC-3'           |
|             | R | 5'-AATCATAAGGCGGGGCTGTG-3'             |
| N-cadherin  | F | 5'-CAACGGGGACTGCACAGATG-3'             |
|             | R | 5'-TGTTTGGCCTGGCGTTCTTT-3'             |
| Vimentin    | F | 5'-AAAGTGTGGCTGCCAAGAACCTGC-3'         |
|             | R | 5'-ACTCAGTGGACTCCTGCTTTGCCT-3'         |
| Snail       | F | 5'-ACCACTATGCCGCGCTCTT-3'              |
|             | R | 5'-GGTCGTAGGGCTGCTGGAA-3'              |
| GAPDH       | F | 5'-CCATGGGGAAGGTGAAGGTC-3'             |
|             | R | 5'-ACATGTAAACCATGTAGTTGAGG-3'          |
| SHIP2       | F | 5'-GCCGGAGGCTGCACAGTGAG-3'             |
|             | R | 5'-CCTCAAAGGCCTGCACGGGG-3'             |
| HBx         | F | 5'-GTTAAGCTTATGGCTGCTAGGCTGTGCTGC-3'   |
|             | R | 5'-AGACTCGAGCCGGGCAGAGGTGAAAAAGTTGC-3' |
| Glut-1      | F | 5'-CGGGCCAAGAGTGTGCTAAA-3'             |
|             | R | 5'-TGACGATACCGGAGCCAATG-3'             |

| Name          | Sequence                    |
|---------------|-----------------------------|
| shLuc         | 5'-ATCACAGAATCGTCGTATGCA-3' |
| shSHIP2#09    | 5'-CCTGAACTACATCAGCAGGAA-3' |
| shSHIP2#10    | 5'-CCGGATTCTGTGGAAATCCTA-3' |
| shSKP2#77     | 5'-GATAGTGTCATGCTAAAGAAT-3' |
| shSKP2#86     | 5'-GCCTAAGCTAAATCGAGAGAA-3' |
| HBX siRNA     | GGUUAAAGGUCUUUGUAUU         |
| Control siRNA | GAUCAUACGUGCGAUCAGA         |

Table S2. Sequences for SHIP2, SKP2, and HBx silencing.