Supplemental Tables and Figure legends

Title: Supplementary Figure 1

Caption: Effects of restoring HIC1 expression in MDA-468 cells.

(A) Restoration of HIC1 expression was confirmed by western blotting and real-time PCR.

(B) HIC1 expression impaired cancer cell invasion ability. Representative images of invaded cells are shown. Mean ± SD. Scale bar, 200 μm.

(C) Effect of HIC1 expression on cell migration in MDA-468 cells. The scratch wound healing assay indicated that HIC1 did not significantly affect cell migration.

Title: Supplementary Figure 2

Caption: LCN2 does not affect the ERK and NF-κB pathways.

Breast cancer cells were stimulated with LCN2 (200 ng/ml) for different times. Whole cell lysates were separated on SDS-PAGE and immunoblotted with different antibodies. The results suggested that exogenous LCN2 stimulation does not affect the ERK1/2 and NF-κB signaling pathways.

Title: Supplementary Figure 3

Caption: HIC1 and LCN2 do not affect epithelial-mesenchymal transition (EMT).

(A) RT-PCR indicated that restoring HIC1 expression in MDA-231 and MDA-468 cells did not affect E-cadherin and vimentin expression at the mRNA level.

(B) Inactivation of endogenous HIC1 expression by shRNA did not affect E-cadherin and
vimentin expression at the protein level.

(C) Stable infected cell lines (1#, 2#, 3# and 4#) were generated as described in Fig. 5B. Western blotting showed that HIC1 and LCN2 expression both had little effect on the expression of E-cadherin and vimentin.

(D) Knockdown of endogenous LCN2 secretion did not affect EMT in MDA-231 cells.

Title: Supplementary Figure 4

Caption: LCN2 does not affect MMP9 expression.

(A) Overexpression of LCN2 was confirmed by real-time RT-PCR and ELISA in MDA-231 cells.

(B) LCN2 overexpression did not affect MMP9 expression at the mRNA level.

(C) Western blot analysis indicated that restoring HIC1 expression did not affect MMP9 expression in conditioned medium (lane 1 and 2). Overexpression of LCN2 did not induce MMP9 expression (lanes 3 and 4). Conditioned medium were collected from 48 h cell cultures with 1% FBS.

Title: Supplementary Figure 5

Caption: LCN2 promotes lung metastasis in \textit{balb/c} nude mice.

(A) Luciferase tagged MDA-231^{PMSCV} and MDA-231^{LCN2} cells were respectively injected into the tail veins of \textit{balb/c} nude mice. At 6 weeks, imaging was performed with the Xenogen IVIS imaging system. Representative images are shown (left, middle). Quantification of tumor burden was performed by bioluminescence imaging (right).
The results showed that lung metastases were increased in the MDA-231\textsuperscript{LCN2} group compared with the control MDA-231\textsuperscript{PMSCV} group. N=5, mean ± SEM.

(B) Quantification of lung metastatic nodules.

(C) Representative images of hematoxylin and eosin staining for lung micrometastasis. Scale bar, 500 μm.

Title: Supplementary Figure 6
Caption: The AKT signaling pathway is partly inactivated after NGALR knockdown.

(A) The efficiency of knockdown by siRNAs in MDA-468 cells was validated by real-time RT-PCR.

(B) The efficiency of knockdown by siRNAs in MDA-231 cells was validated by real-time RT-PCR.

(C) Inactivation of NGALR in MDA-468 cells by shRNAs. The knockdown efficiency was confirmed by real-time RT-PCR.

(D) The AKT signaling pathway was partly inactivated after NGALR knockdown in cells with exogenous LCN2 stimulation at 0, 30, 60 min.

Title: Supplementary Figure 7
Caption: LCN2 does not affect PTEN activity.

(A) Knockdown of endogenous LCN2 by siRNAs did not affect PTEN activity in MDA-231 cells.

(B) Exogenous LCN2 stimulation did not affect PTEN activity in MDA-231 and MDA-468
Title: Supplementary Figure 8

Caption: Endogenous HIC1 knockdown promotes cell migration and invasion in MCF-10A cells.

(A) The efficiency of HIC1 knockdown by a combination of shHIC1-2 and 3 was confirmed by western blotting.

(B) HIC1 knockdown in MCF-10A cells greatly increased their invasive ability as compared with the control. Representative images of invaded cells are shown. Mean ± SD. Scale bar, 200 μm.

(C) HIC1 knockdown in MCF-10A cells greatly increased their migration ability as compared with the control. BD Falcon cell culture inserts (8 μm) were used for the cell migration assay (Cat#: 353097, BD). Representative images of migrated cells are shown. Mean ± SD. Scale bar, 200 μm.

Title: Supplementary Table 1

Caption: Correlation between expression of HIC1 and clinical pathological features in tissue microarrays.

A tissue microarray (BR1921, BIOMAX.US) was stained with anti-human HIC1 antibody (1:200, H8539, Sigma). The staining intensity was scored on a scale of five as follows: negative (-), negligible (±), weak (+), moderate (++) and strong (+++). Negative (-) and negligible (±) staining for HIC1 were defined as the negative group, and the others were
defined as the positive group. Pearson’s Chi-Square tests were used to analyze the correlation between variables.

**Title: Supplementary Table 2**

**Caption: Serum levels of LCN2 in breast cancer patients.**

Serum was collected from patients before surgery and serum LCN2 level was detected by ELISA. Before detection, the serum samples were diluted 1:50 with 1% BSA. ELISA was performed according to the manufacturer’s instructions. The Student’s t test was used to determine the statistical significance of differences between groups.