Supplementary information:

Journal of Hepatology (2014)

Endoplasmic reticulum heat shock protein gp96 maintains liver homeostasis and promotes hepatocellular carcinogenesis

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Four figures.

Supplementary Figure 1
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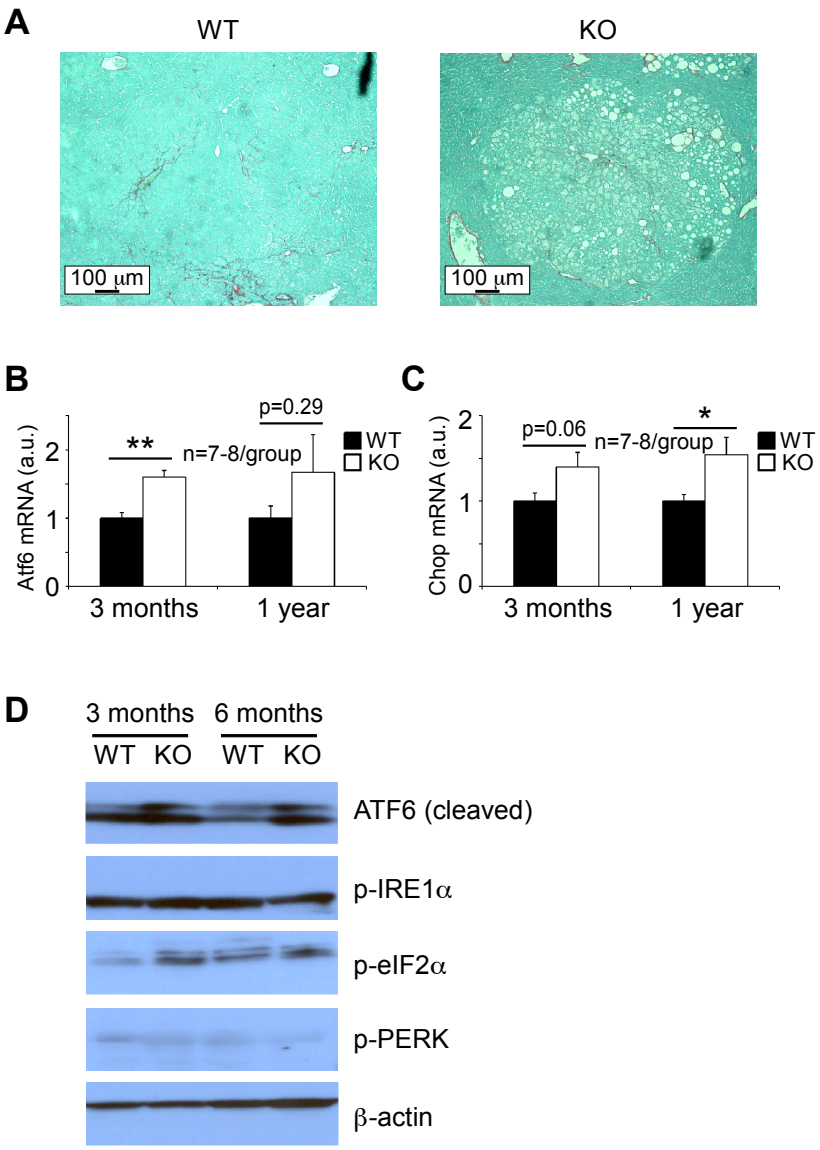

Supplementary Figure 1. gp96 KO livers display no fibrosis and moderate levels of ER stress at baseline. (**A**) WT and KO livers from 3 month-old males were stained with picrosirius red for liver fibrosis. (**B,C**) qRT-PCR for ATF6 (**B**) and CHOP (**C**) on whole liver tissue from 3 month-old WT and KO mice (n=8 per group). (**D**) Immunoblot of whole liver cell lysates from 3 month and 6 month-old mice of indicated proteins (representative of 4 mice per group).

Supplementary Figure 2
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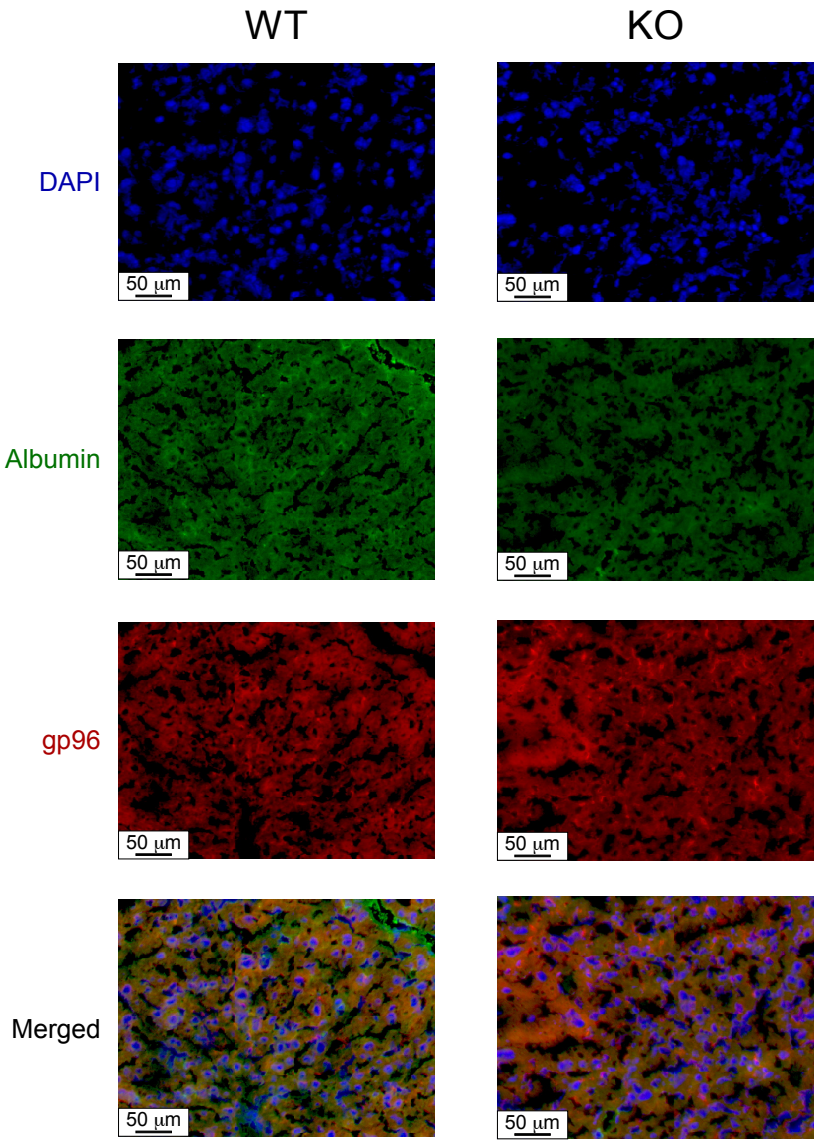

Supplementary Figure 2. Tumor cells from both WT and Albumin-cre gp96 KO mice are gp96+ hepatocytes. Sections of liver lesions from diethylnitrosamine (DENA)-treated mice 32 weeks post-injection were co-stained by immunofluorescence for albumin and gp96, and counterstained of nuclei with DAPI (diamidino-2-phenylindole).

Supplementary Figure 3
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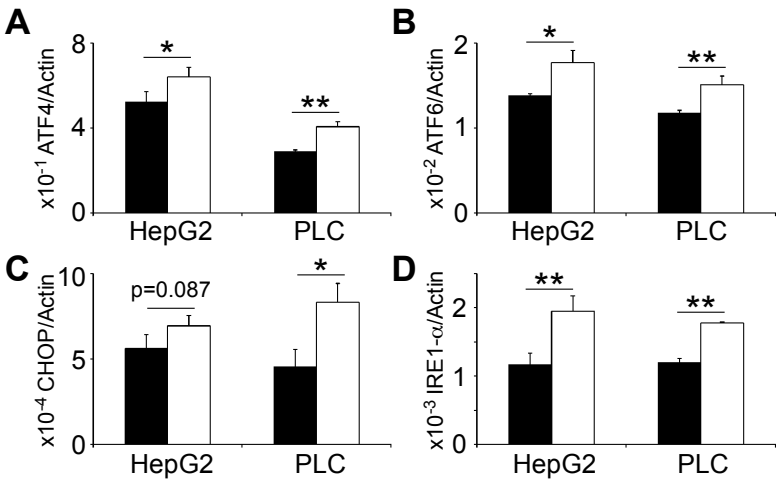

Supplementary Figure 3. ER stress in hepatoma cells upon gp96 inhibition. qRT-PCR for ATF4 (**A**), ATF6 (**B**), CHOP (**C**) and IRE-1α (**D**) in PLC and HepG2 cells treated with 2 μM (PLC) or 6 μM (HepG2) WS13 for 36 hours. Error bars represent standard deviation.

Supplementary Figure 4
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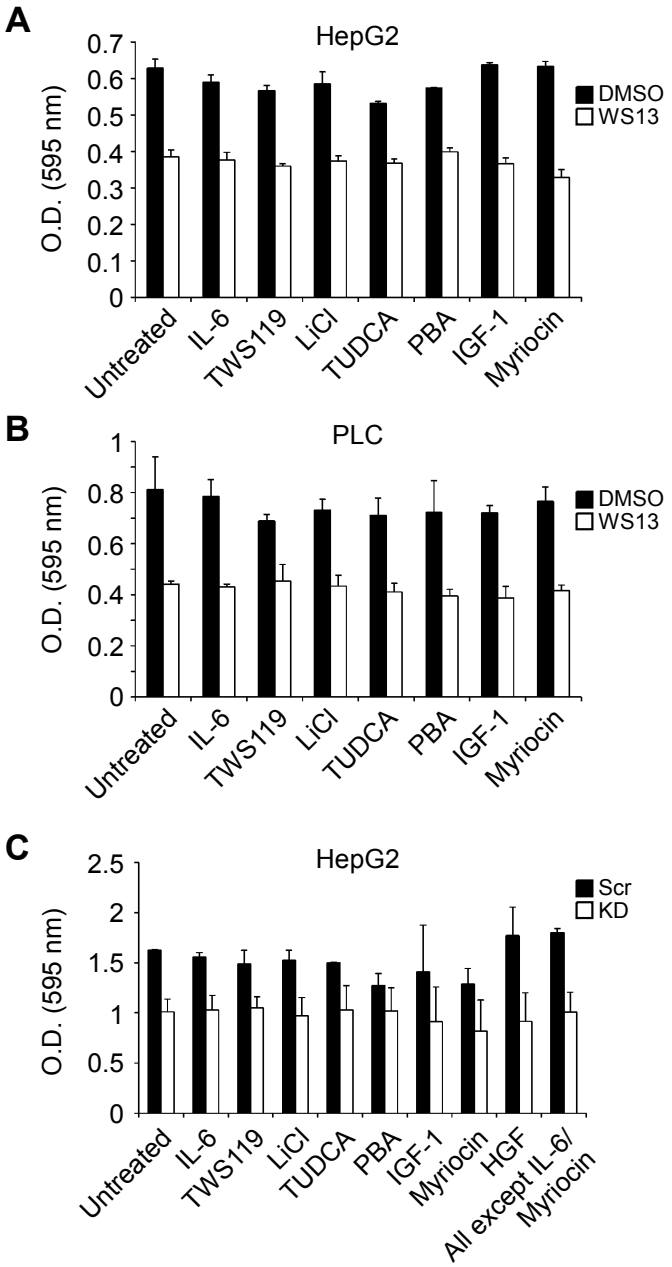

Supplementary Figure 4. Rescue of ER homeostasis, Wnt signaling, IL-6 or IGF-1 or inhibition of *de novo* ceramide synthesis are not sufficient to restore cellular proliferation defect in response to selective gp96 inhibitor. (**A,B**) HepG2 (**A**) and PLC (**B**) cells were treated with 20 μM WS13 for 3 days, with or without IL-6, TWS119, LiCl, tauroursodeoxycholic acid (TUDCA), 4-phenylbutyric acid (PBA), IGF-1 or myriocin, followed by cell number quantification using MTT assay. (**C**) Scrambled shRNA or gp96 knockdown cells were cultured for 3 days with or without IL-6, TWS119, LiCl, tauroursodeoxycholic acid (TUDCA), 4-phenylbutyric acid (PBA), IGF-1, myriocin, hepatocyte growth factor (HGF) or a combination of them, followed by cell quantification using MTT assay.