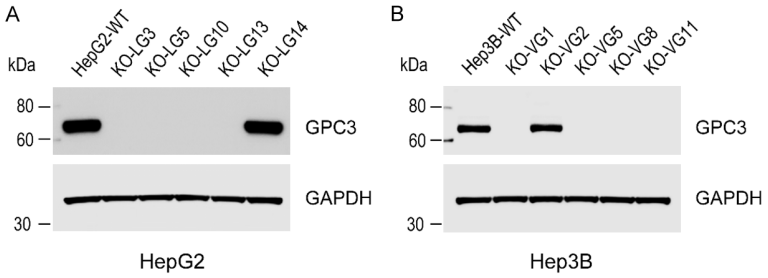
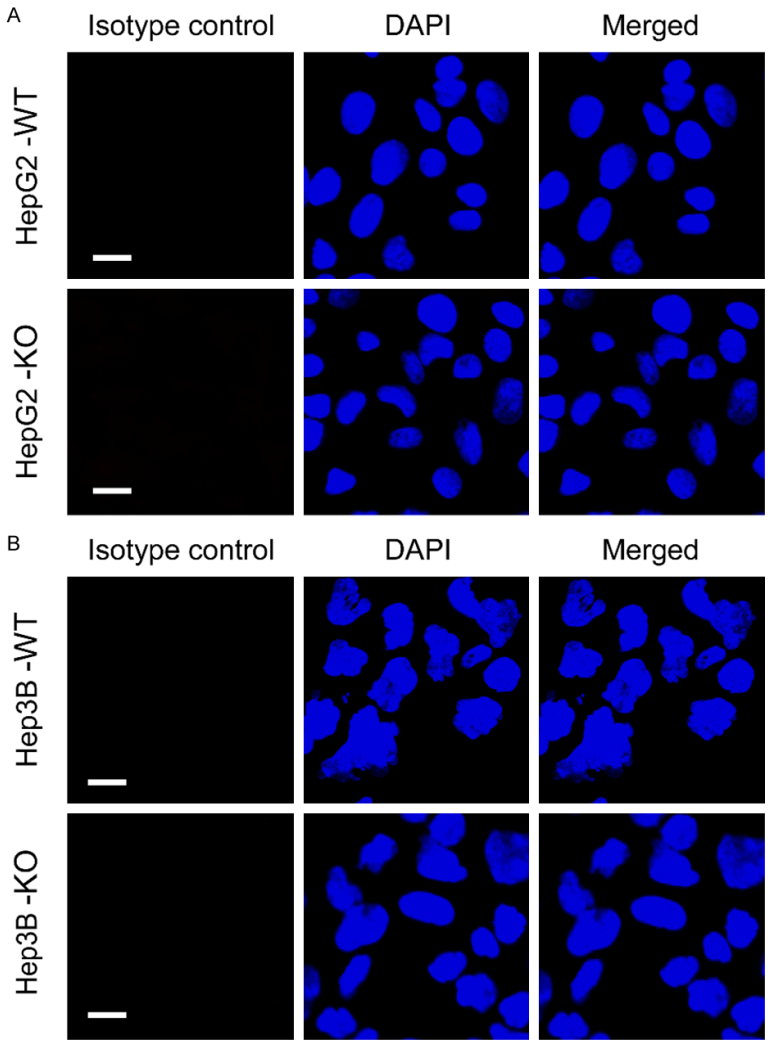
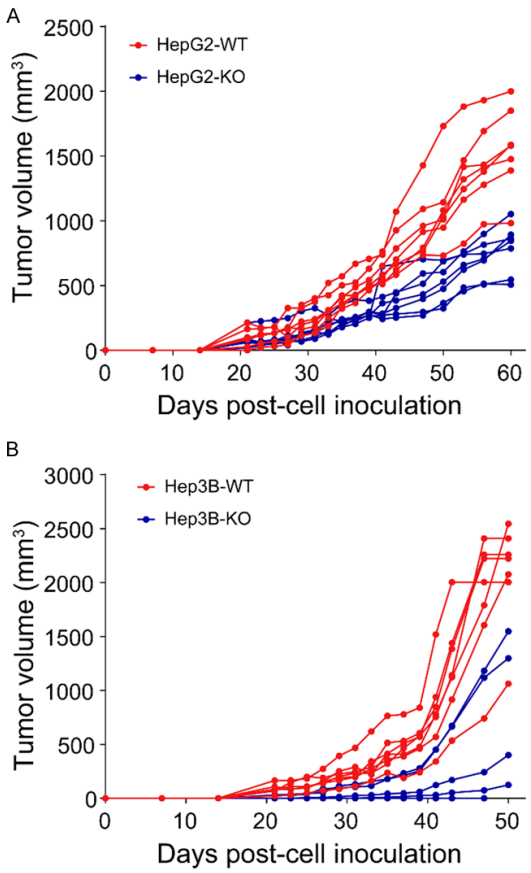


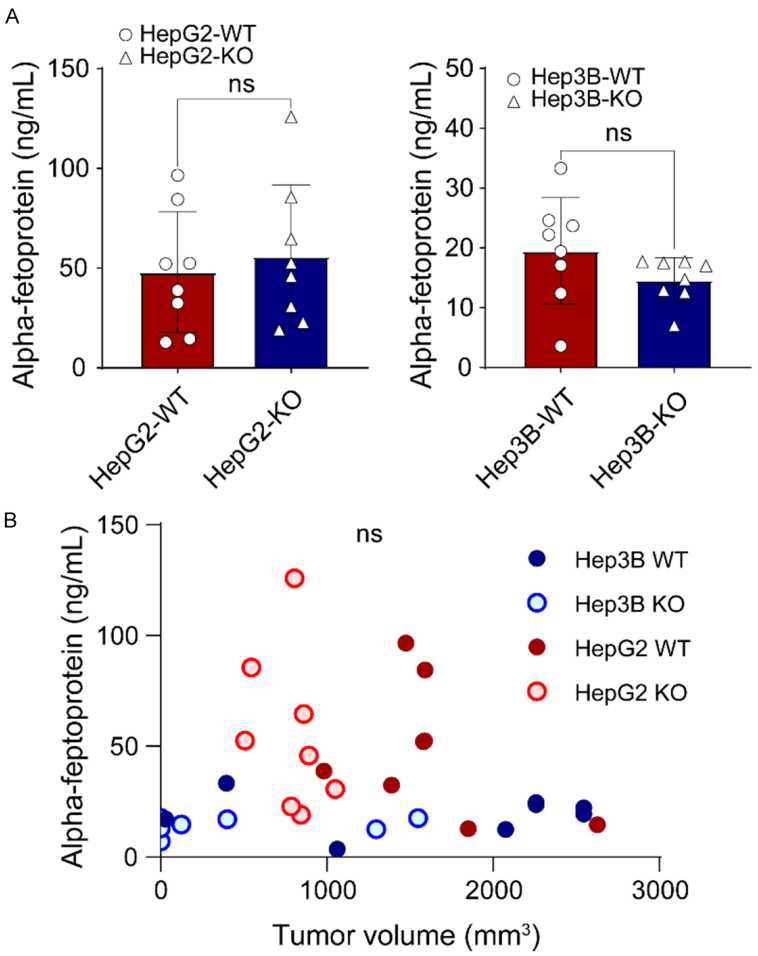
Supplementary Figure 1. Knockout of GPC3 expression in HepG2 and Hep3B cells using CRISPR/Cas9. Single-cell clones KO-LG3 (A) and KO-VG5 (B) were chosen for subsequent analysis in HepG2 and Hep3B, respectively.



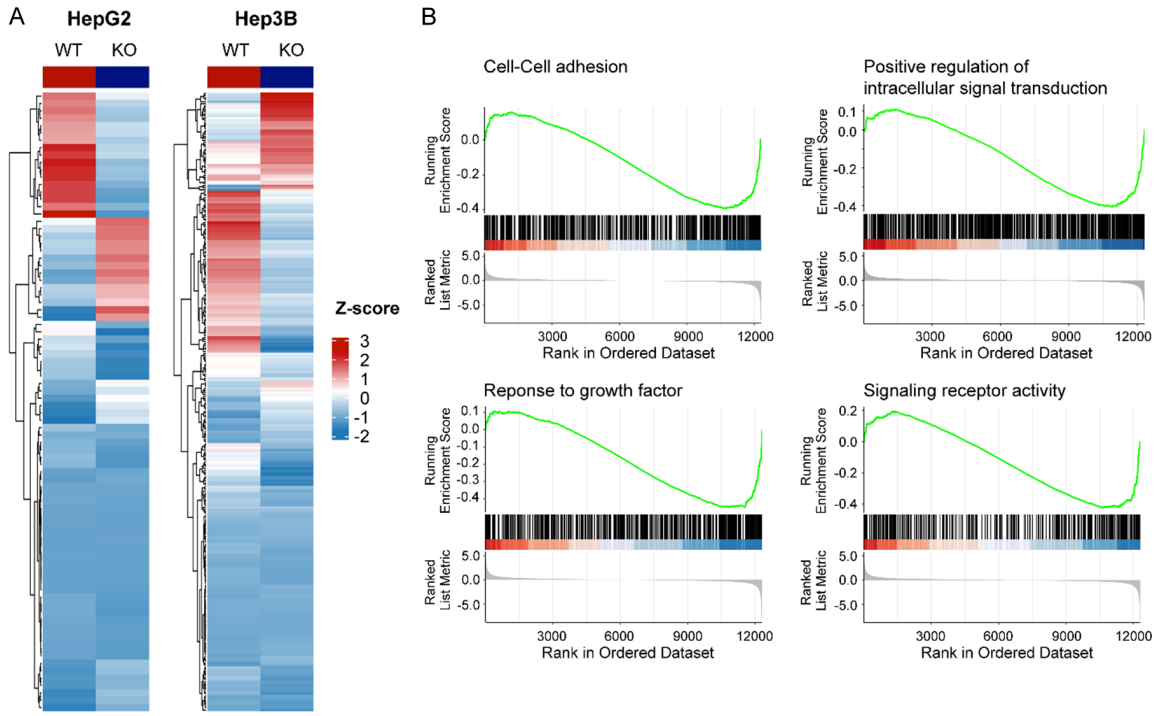
Supplementary Figure 2. Immunofluorescence staining with isotype control. The isotype control was negative in both HepG2 (A) and Hep3B (B) parental cells as well as in cells deficient in GPC3. Scale bars are shown for 20 μm.



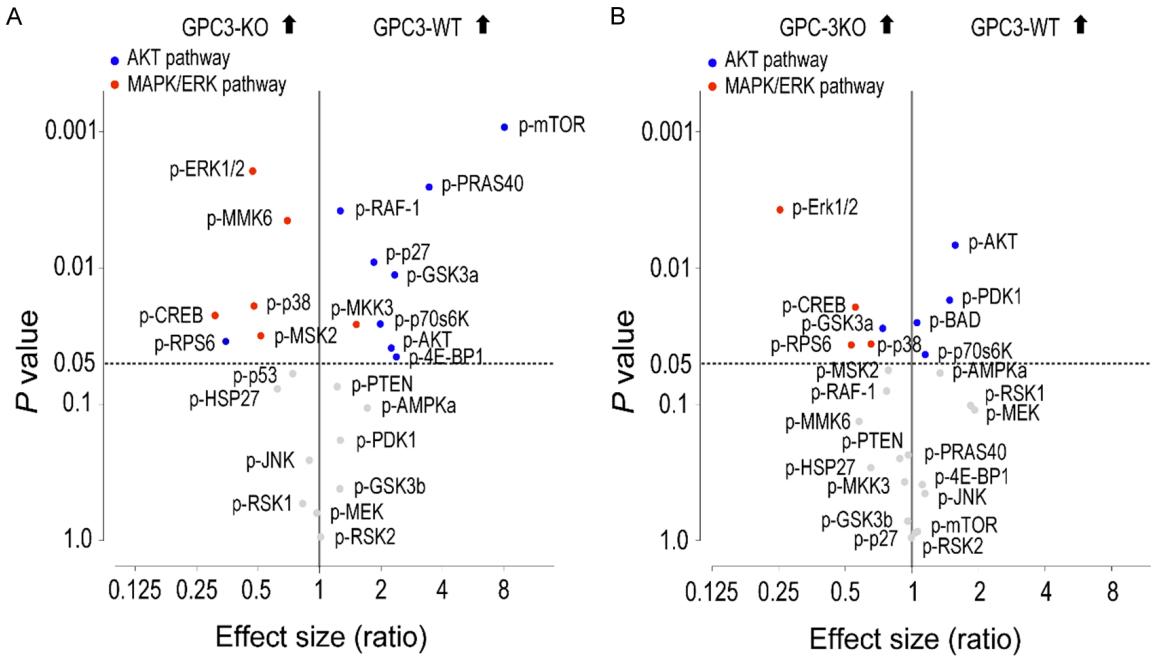
Supplementary Figure 3. Tumor volume growth curves for individual mice with parental and GPC3-KO cells. A. Growth curves depicting tumor volume over time for individual mice inoculated with HepG2 WT and GPC3-KO cells. B. Growth curves depicting tumor volume over time for individual mice inoculated with Hep3B WT and GPC3-KO cells. Tumors were subcutaneously inoculated into BALB/c nu/nu mice, and tumor volumes were estimated based on caliper measurements conducted three times per week throughout the course of tumor growth.



Supplementary Figure 4. Association between alpha fetoprotein and GPC3. A. Serum alpha fetoprotein (AFP) levels in xenograft tumors derived from HepG2 and Hep3B wild type and GPC3 knockout cells. B. Correlation between serum AFP levels and tumor volume.



Supplementary Figure 5. Effects of GPC3 knockout on gene expression profiles in HepG2 and Hep3B cells. A. Heatmap representation of differentially expressed genes upon GPC3-KO, relative to parental liver cancer cells. Expression values of genes are scaled by row. B. GSEA plots of top enriched GO BP gene sets downregulated in Hep3B cells.



Supplementary Figure 6. Phospho-AKT and Phospho-MAPK/ERK array analysis of parental and GPC3 knockout liver cancer cells. Protein extracts (500 µg each) were prepared from whole-cell lysates of both WT and GPC3-KO cultures of HepG2 (A) or Hep3B (B) cells. These extracts were then subjected to analysis using the phosphorylated protein array (Raybiotech) for analysis. Array spots were visualized according to the manufacturer's instructions. The intensity of each array spot was measured in arbitrary units using the ChemiDoc MP Imaging system (Bio-Rad) and analyzed with ImageQuant 5.2 (Molecular Dynamics Inc.). The resulting plots depict the relative fold change of phosphorylated AKT (in blue) and MAPK/ERK (in red) signaling molecules against the logarithm of their *p*-values. Volcano plots were generated through a multiple t-test comparison between WT and GPC3-KO conditions, employing Prism (ver. 10, GraphPad Software) for statistical analysis.