Supporting Information

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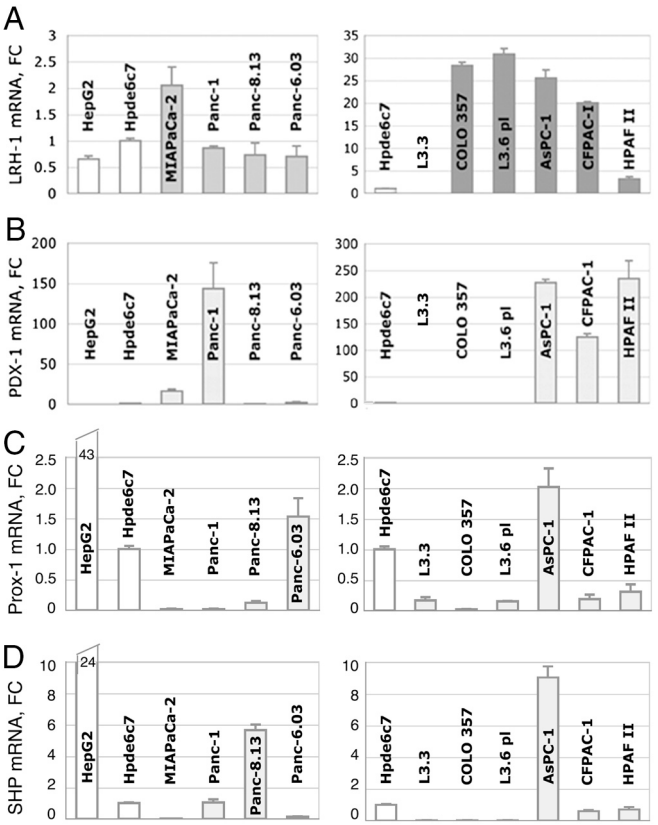

Fig. S1. Expression of liver receptor homologue 1 (LRH-1) and its regulators in pancreatic cancer cells. (*A*) The levels of LRH-1 mRNA in different pancreatic cancer cells. Levels of LRH-1 specific mRNA in pancreatic cancer cell originated from primary and metastatic pancreatic ductal adenocarcinoma tumors (light- and dark-gray bars in the left and right panels, respectively) are shown as fold change (FC) relative to that in nonneoplastic pancreatic ductal epithelial cells Hpde6c7 (control in white). For comparison, the levels of LRH-1 mRNA in hepatocarcinoma cells HepG2 are shown in the left panel. Standard deviations are drawn as black lines. (*B–D*) Quantitative PCR data indicating the levels of specific mRNA for pancreatic and duodenal homeobox 1 (PDX-1, *B*), the Prospero homeobox protein 1 (Prox1, *C*), and the short heterodimer partner (SHP, *D*) in pancreatic cancer cells. The corresponding levels for each specific mRNA are shown as gray bars, relative to the control (immortalized pancreatic ductal epithelial cells Hpde6c7, indicated).

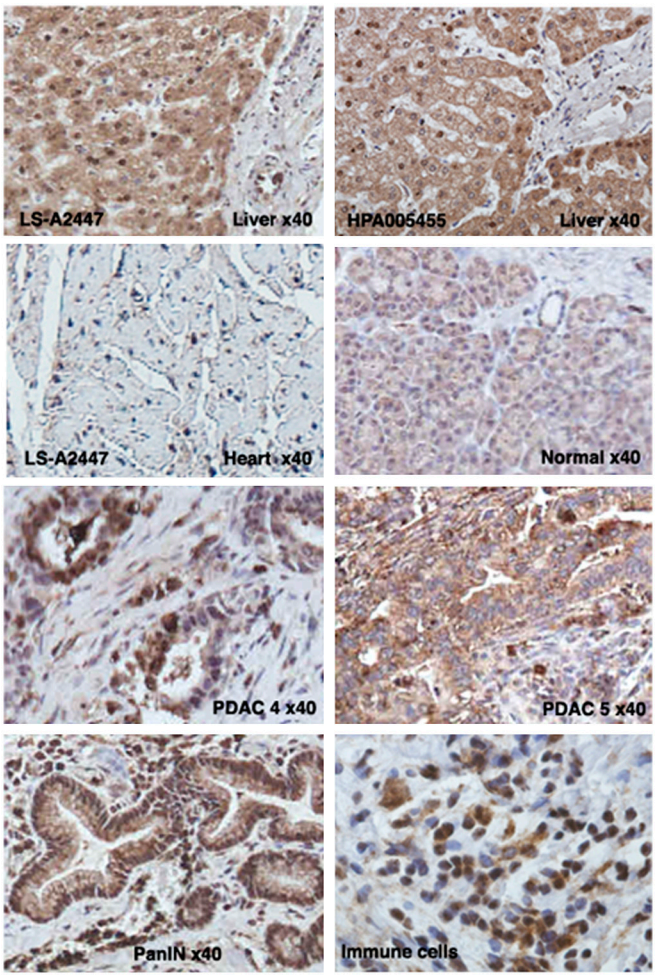

Fig. S2. Differential expression of liver receptor homologue 1 (LRH-1) in normal and cancerous pancreas. Shown are representative immunohistochemistry images of human normal (indicated Normal) and neoplastic pancreas [pancreatic ductal adenocarcinoma (PDAC) indicated as PDAC 4–PDAC 5]. The samples were treated with primary antibody LS-A2447 (LifeSpan Biosciences), stained with ImmPACT diaminobenzidine (Vector Labs), and counterstained with hematoxylin; the original magnification (×40) is specified. The specificity of staining [both for LS-A2447 and HPA005455 (Sigma-Aldrich) antibodies, indicated] was confirmed using positive (liver hepatocytes, with positively stained nuclei and cytoplasm) and negative (liver connective tissue) controls (Liver, indicated). An additional negative control (heart muscle tissue sample; Heart, indicated) showed the absence of unspecific background staining. Weak cytoplasmic and weak-to-moderate punctate nuclear staining is observed for all components of normal exocrine pancreas. In neoplastic cells, elevated levels of LRH-1 protein are observed either in the nuclei (PDAC 4) or in the cytoplasm (PDAC 5) or both. The fibroblasts in the stroma surrounding the neoplastic lesions do not show significant LRH-1 specific staining (PDAC 4), however, many infiltrating immune cells, including lymphocytes and neutrophils, are positively stained for LRH-1 (Immune cells, indicated; original magnification ×40; image is additionally magnified for clarity). Heightened levels of LRH-1 are also observed in pancreatic intraepithelial neoplasia (PanIN) lesions (indicated). We note that, although the receptor quantification in cultured cancer cells did not reveal significant differences in the levels of LRH-1 between nonneoplastic and primary tumor cells (Fig. 1 of the manuscript), the immunohistochemistry (IHC) evaluations of primary PDACs demonstrated apparent increases in the receptor levels in all tested tumor samples (12 PDAC cases, 100%) compared to normal exocrine pancreas (Fig. 2, Fig. S2, and Table S1). A few contributing factors might account for these contradicting data. First, cultured pancreatic cancer cells have been shown to have nonidentical gene expression profiles compared to the surgical PDAC specimens. Second, the control Hpde6c7 cells used for in vitro receptor quantification are not equivalent to normal pancreatic ductal epithelium because these cells are immortalized and thus have a plenitude of genes (including those that control cell renewal and proliferation) activated. As LRH-1 is connected intimately to these processes, modest differences observed between the control and the primary cancer cells might be attributed to a heightened LRH-1 activity in Hpde6c7 cells. In this regard, evaluation of LRH-1 expression in PDAC tumors is superior to the receptor quantification in cultured cancer cells. The IHC analysis of PDACs has an additional advantage of revealing differential LRH-1 expression in various types of neoplastic cells as well as in different cancer cell compartments. Further in-depth studies are needed to reveal possible variations in LRH-1 expression in different subtypes of PDACs. This information might be invaluable for development of pancreatic cancer theranostics. The potential of theranostic strategies for cancer treatments is best exemplified by the success of human epidermal growth factor receptor 2 (HER2)-based diagnostics and the receptor targeting therapies that extend life of many breast cancer patients.

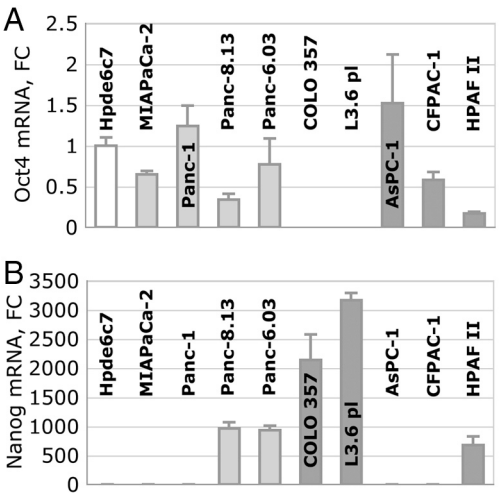

Fig. S3. Evaluation of the liver receptor homologue 1 (LRH-1) target genes controlling cell differentiation. (*A* and *B*) Quantitative PCR data showing levels of mRNA for Oct4 (*A*) and Nanog (*B*) in pancreatic cancer cells. Levels of specific mRNA in primary and metastatic cells are shown as fold change (FC) in light and dark gray, relative to the corresponding control levels in normal pancreatic cells Hpde6c7. Standard deviations are drawn as black lines.

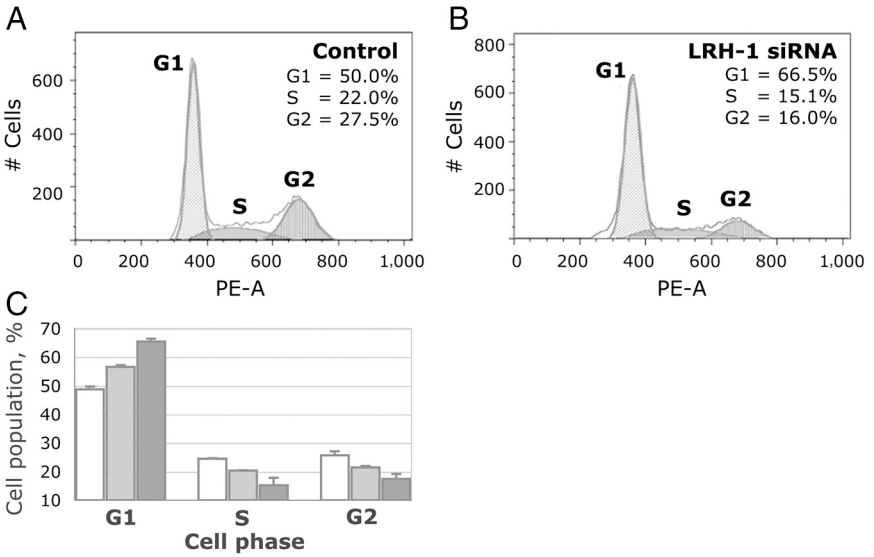

Fig. S4. Effects of blocking of liver receptor homologue 1 (LRH-1) on cell cycle. (*A* and *B*) Fluorescence-activated cell sorting analysis of AsPc-1 cells transfected with irrelevant (*A*) and anti-LRH-1 (*B*) siRNAs. Cells treated with either irrelevant or specific anti-LRH-1 siRNAs were fixed, stained with propidium iodide, and analyzed using BD LSR II flow cytometer. The cell cycle profiles were analyzed with FlowJo software. The populations of cells in G1, S, and G2 phases are characterized by different intensities of R-phycoerythrin (PE-A) fluorescence and are indicated. (*C*) Specific inhibition of LRH-1 by siRNA affects the cell cycle G1/S transition. The histogram shows cumulative data on distribution of cells treated with siRNA between different phases of the cell cycle (indicated). Control in white corresponds to cells transfected with irrelevant siRNA, light- and dark-gray bars indicate cells transfected with two different anti-LRH-1 siRNAs. Data were measured on day 2 following transfections with siRNAs, when the levels of both LRH-1 and its transcriptional targets are minimal.

Other Supporting Information Files

Table S1 (DOC)