Figure S1. The RNAi-mediated knockdown of PIM2 in HepG2 and Huh-7 liver cancer cell lines. (A) Knockdown of PIM2 by three different siRNAs (siPIM2A, siPIM2B, siPIM2C) on the mRNA level (n=3) 48 h post-transfection, as analyzed by RT-qPCR. (B) Analysis of siRNA-mediated of PIM2 knockdown by siPIM2A-siPIM2C, as determined at the protein level by western blot analysis at 72 h following transfection. Representative blots are shown. $^{**}P<0.01$ and $^{***}P<0.001$, significant differences compared to the siCtrl. PIM2, proviral integration site for Moloney murine leukemia virus 2.

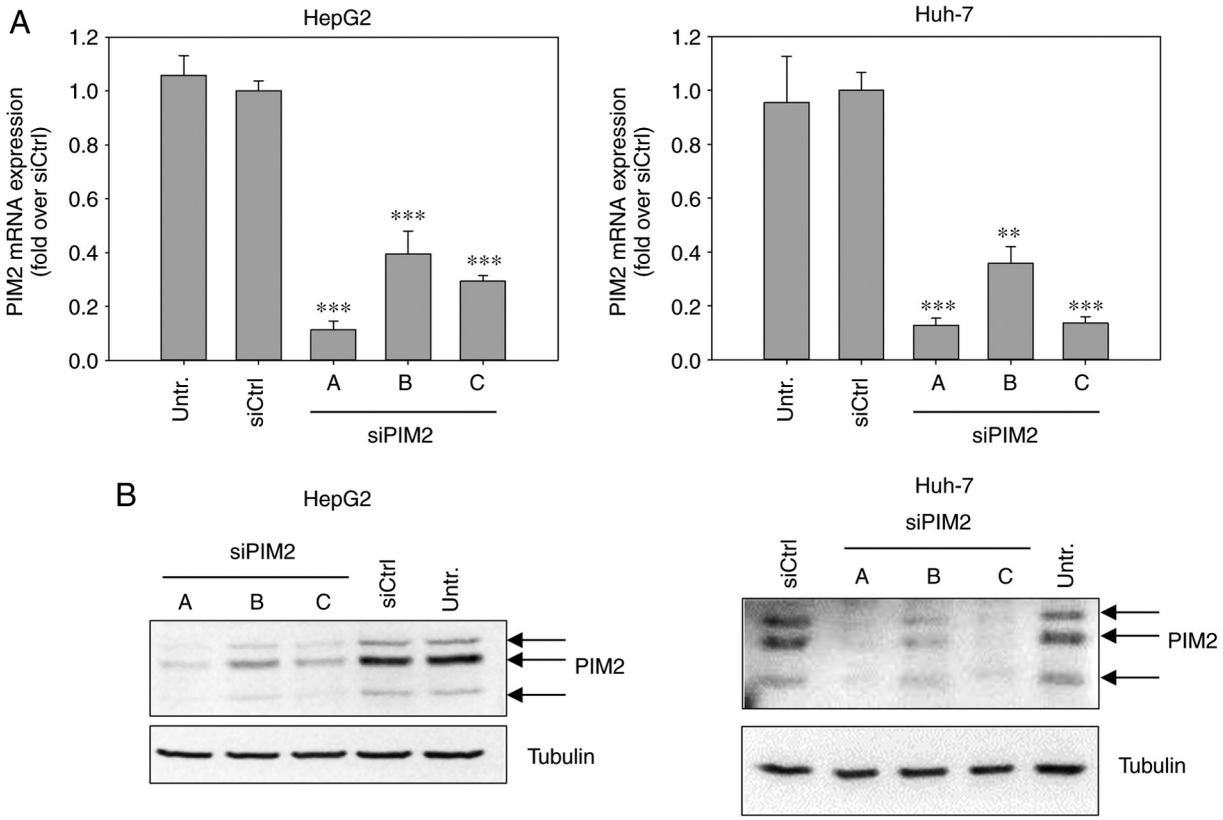

Figure S2. Influence of the knockdown of PIM2 on primary colony density of HepG2 and Huh-7 liver cancer cell lines. Colony spread assay was performed as described in the Materials and methods in the main text and densities of the primary colonies were determined after 7 days (n=4). ***P<0.001, significant differences compared to the siCtrl; the hash symbol (#) indicates that there were no significant differences. PIM2, proviral integration site for Moloney murine leukemia virus 2.

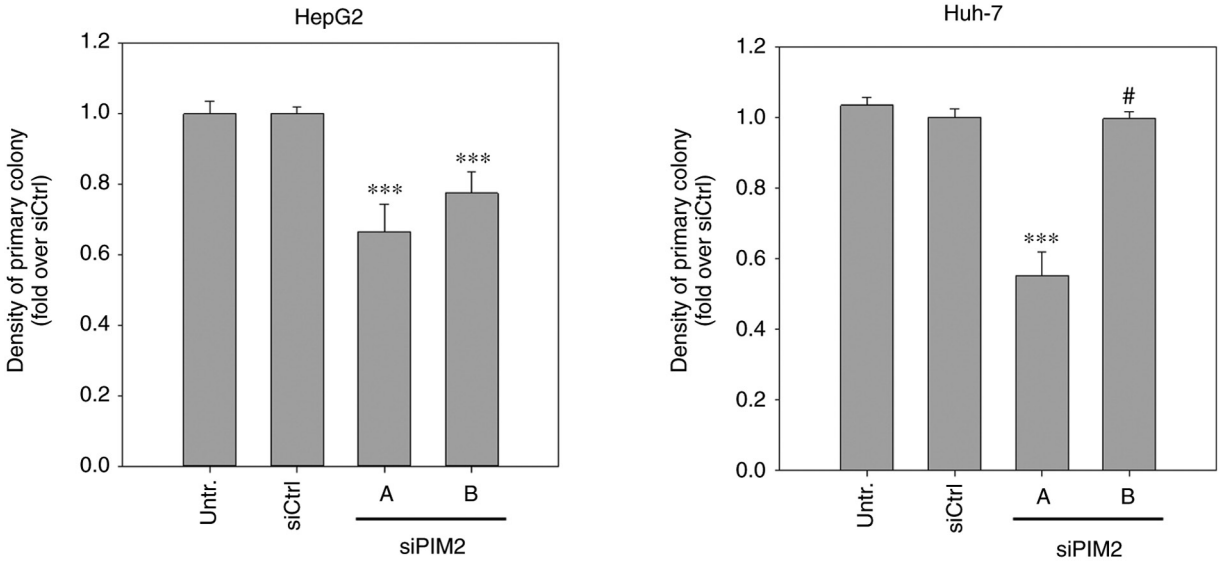

Figure S3. Role of PIM2 knockdown in the apoptosis and death of the liver cancer cell lines, HepG2 (left panel) and Huh-7 (right panel). Representative example of FACS analysis plots of cells stained with Annexin V-FITC and PI at 48 h following transfection (please see Fig. 2A in the main text for mean values of n=3 experiments). PIM2, proviral integration site for Moloney murine leukemia virus 2.

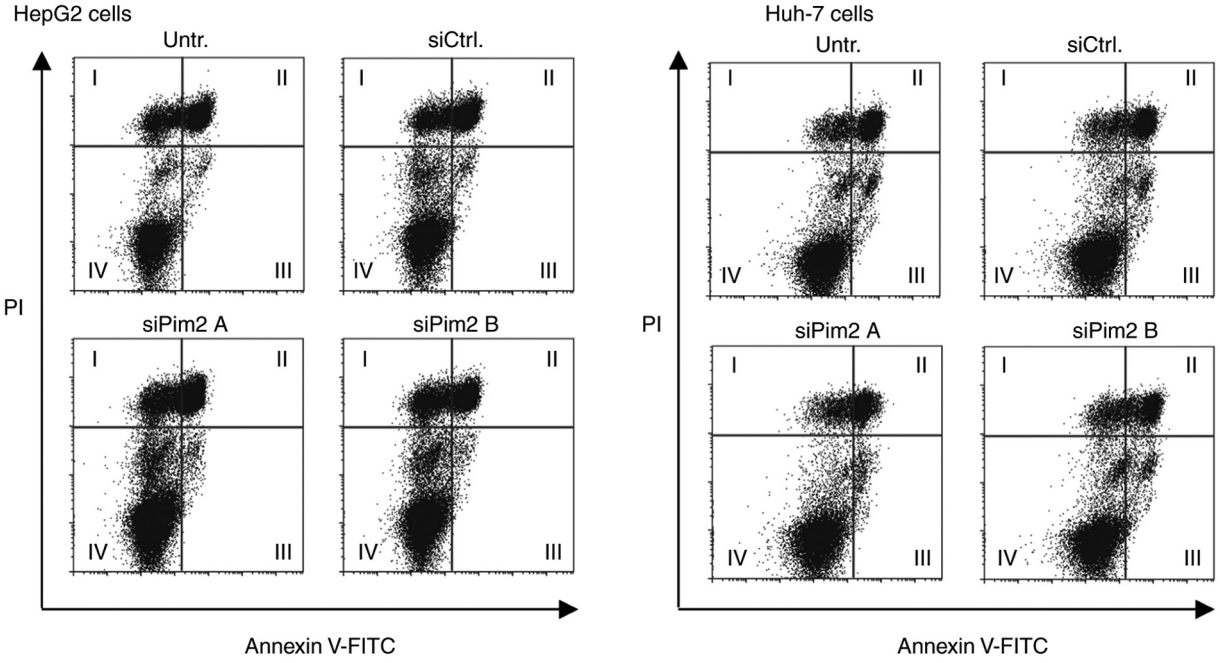

Figure S4. Dependency of Pim2 knockdown-mediated cell cycle effects on $p21^{WAF1/CIP1}$. (A) $p21^{WAF1/CIP1}$ mRNA expression in HepG and Huh-7 cells, as determined by RT-qPCR (n=3). (B) Dependence of PIM2 knockdown-mediated cell cycle alterations on $p21^{WAF1/CIP1}$ levels. Huh-7 cells were transfected with plasmids coding for wildtype $p21^{WAF1/CIP1}$ (p21 wt) or nonfunctional mutated $p21^{WAF1/CIP1}$ (p21 mut) prior to siRNA transfection. After 72 h, the relative percentage of the cell population in the different phases of cell cycle was analyzed by flow cytometry, after nocodazole treatment as described in the text. A representative example of three independent experiments is shown.

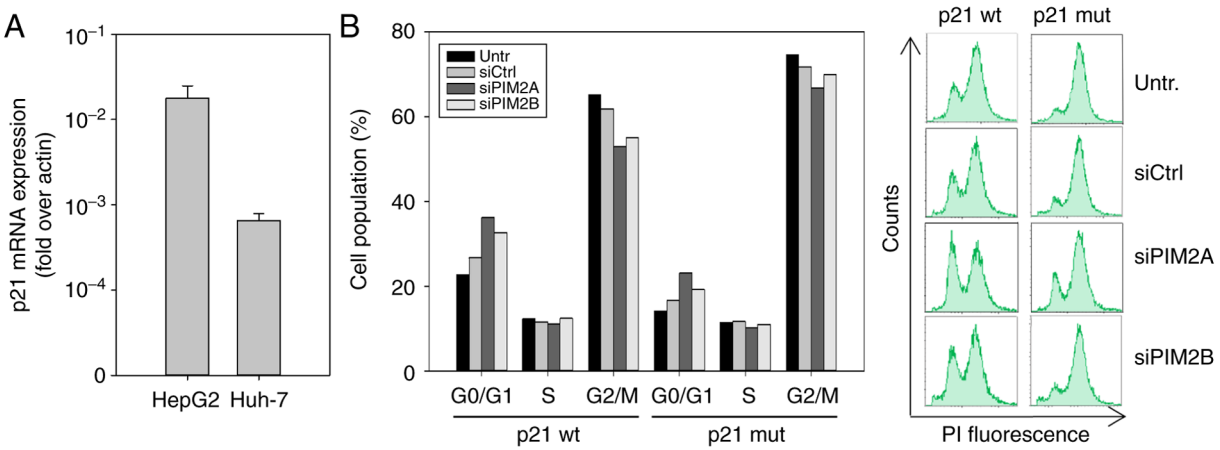

Table SI. Primer sequences.

Target	Forward primer (5'-3')	Reverse primer (5'-3')
PIM2	GTGGCCATCAAAGTGAATTCC	TTCGAGTGGGCATGTGACT
Cyclin A2	AGAAAAAGAAGTCAGAAGAAGCC	ACACTCACTGGCTTTTCATCTT
Cyclin B1	CATGGTGCACTTTCCTCCTT	AGGTAATGTTGTAGAGTTGGTGTC
Cyclin E2	CGAGCGGTAGCTGGTCTGG	CTGCTGCTTAGCTTGTAAACGG
CDK1	CATGGGGATTCAGAAATTGA	ATTCGTTTGGCTGGATCATA
CDK2	AAGCCAGAAACAAGTTGACG	GAAGAGGAATGCCAGTGAGA
CDK4	GAGTGTGAGAGTCCCCAATG	ATGCTCAAACACCAGGGTTA
CDK6	CAGGGGATTTTCATGTTGAG	GCACATCAACGGAATTTTTC
Survivin	TGATGAGAGAATGGAGACAGAG	ACAGCAGTGGCAAAAGGAG
p21	AGCAGAGGAAGACCATGTGGAC	TTTCGACCCTGAGAGTCTCCAG
Actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATA