$ZnAs@SiO_2$ nanoparticles as a potential anti-tumor drug for targeting stemness and epithelial-mesenchymal transition in hepatocellular carcinoma via SHP-1/JAK2/STAT3 signaling

Supplemental Material

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Methods

Primary cells

Following informed consent, fresh tissue samples were obtained from patients undergoing surgery at the Department of Hepatological Surgery, The Fifth Affiliated Hospital, Sun-Yat Sen University. Primary liver cancer cells were derived from tissues of patients who had not yet undergone chemotherapy and were undergoing liver cancer resection. The isolation and culture of primary cells were performed as described previously [1]. Tumor-derived cells were used at passage 1.

Results

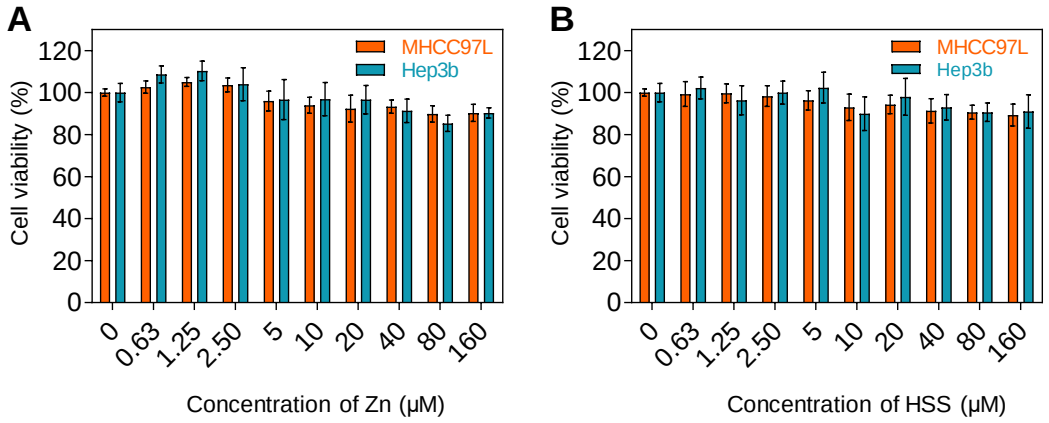

Figure S1. Viability of MHCC97L and Hep3b cells after treatment with various concentrations of $ZnCl_2$ **(A)** or HSS **(B)** for 24 h.

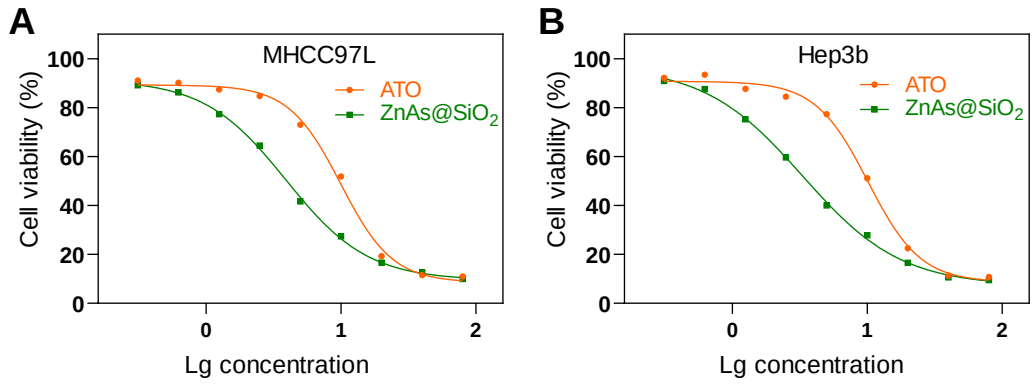

Figure S2. IC_{50} curves of ATO and $ZnAs@SiO_2$ in MHCC97L (A) and Hep3b (B) cells.

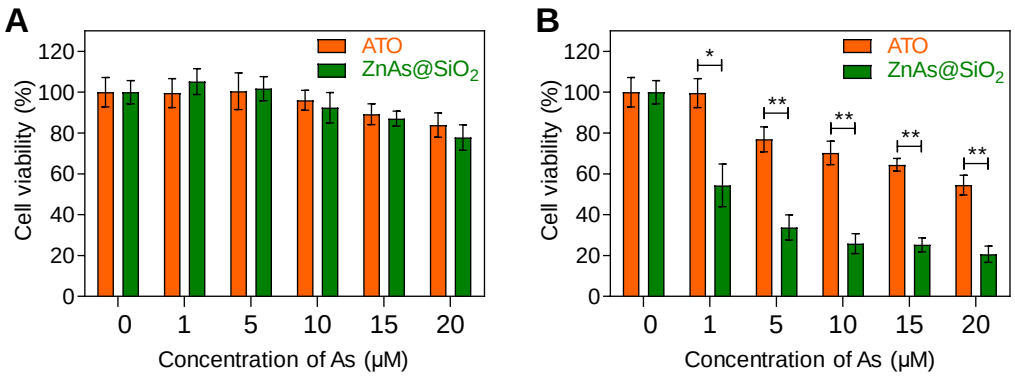

Figure S3. Viability of primary hepatocytes and HCC cells after treatment with various concentrations of ATO or $ZnAs@SiO_2$ NPs for 24 h. *, $P < 0.05$; **, $P < 0.01$.

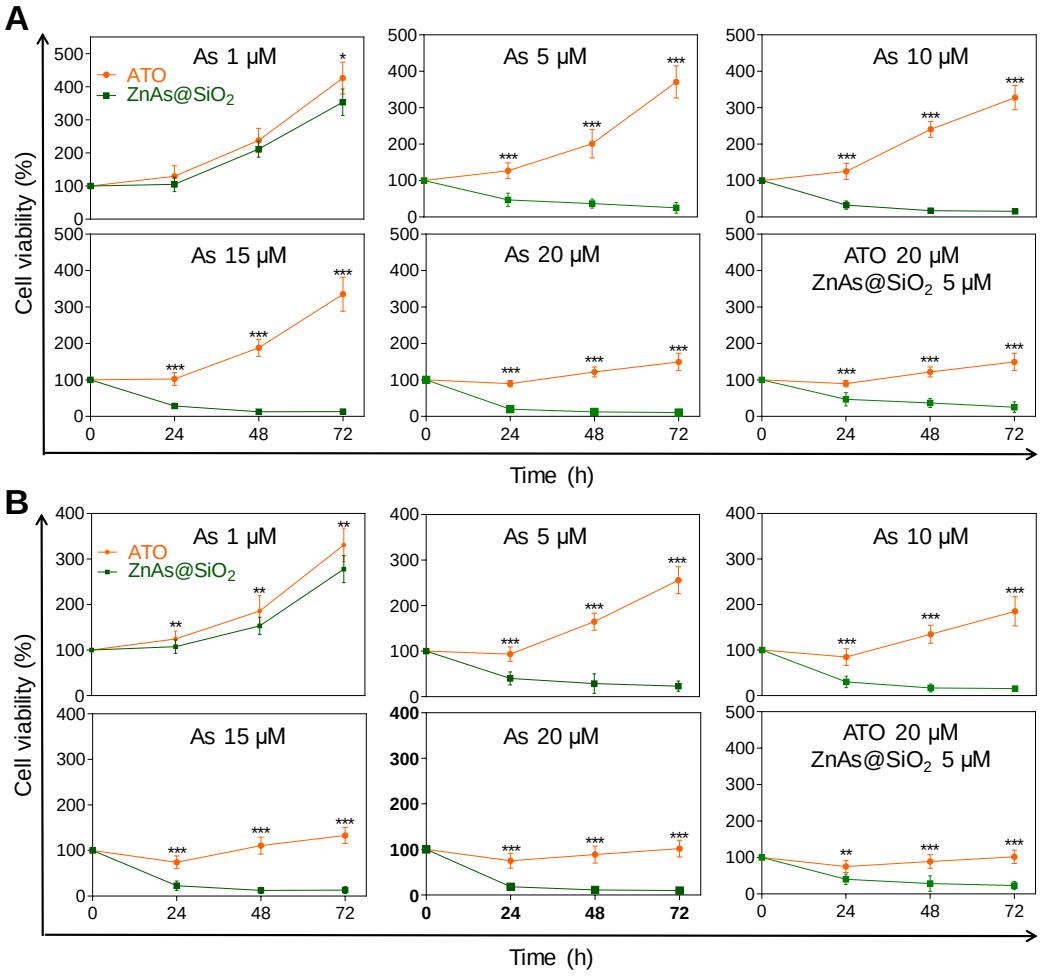

Figure S4. Viability of MHCC97L **(A)** and Hep3b **(B)** cells after treatment with various concentrations of ATO or $ZnAs@SiO_2$ NPs for 24 h, 48 h, and 72 h. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

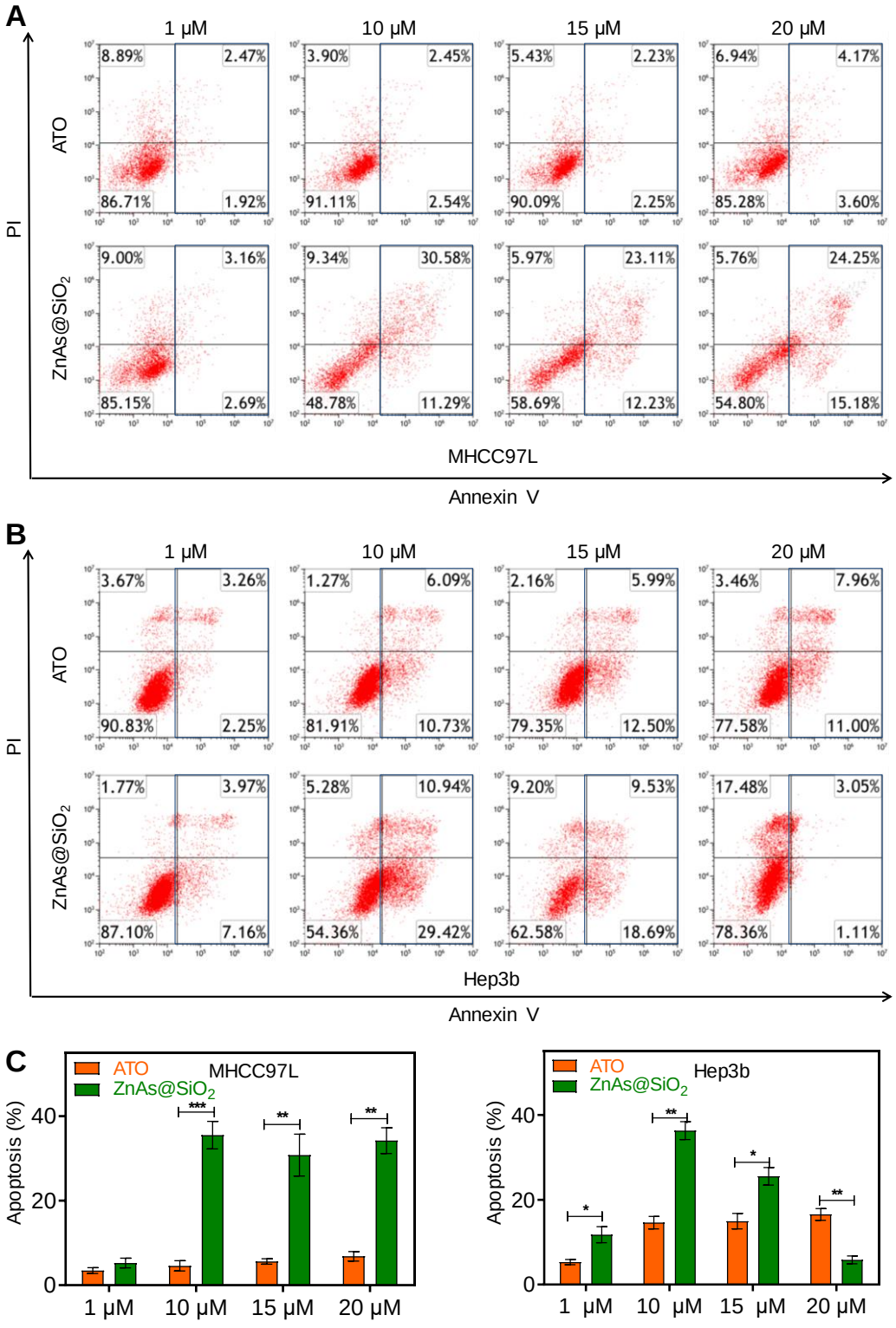

Figure S5. Analysis of apoptosis of MHCC97L **(A)** and Hep3b **(B)** cells and quantification **(C)** after treatment with ATO or ZnAs@SiO_2 NPs at indicated concentrations for 24 h. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

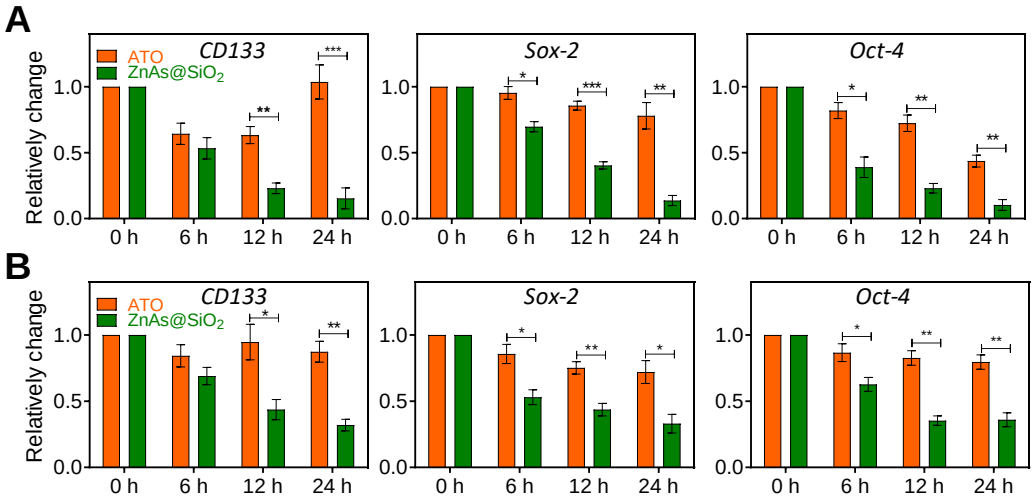

Figure S6. Changes of stemness markers, CD133, Sox-2, and Oct-4 at the mRNA level of MHCC97L **(A)** and Hep3b **(B)** cells after treatment with ATO or ZnAs@SiO_2 NPs at indicated time points. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

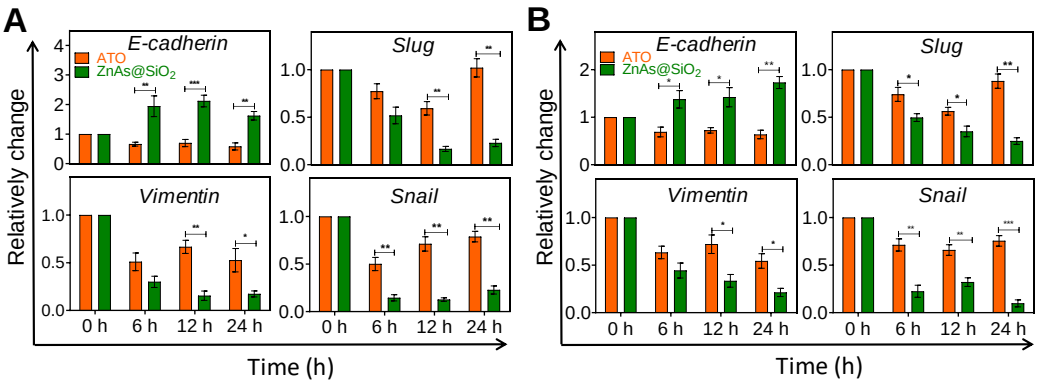

Figure S7. Changes of EMT markers, E-Cadherin, Snail, Slug, and Vimentin at the mRNA level in MHCC97L **(A)** and Hep3b **(B)** cells after treatment with ATO or ZnAs@SiO_2 NPs at indicated time points. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

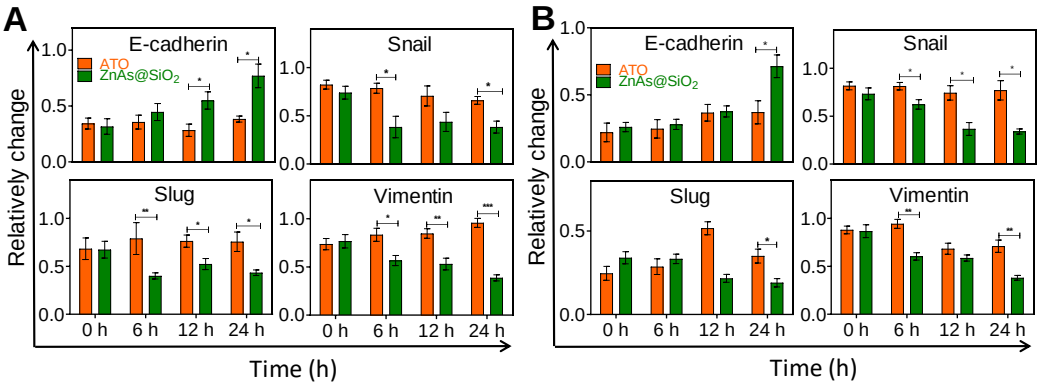

Figure S8. Quantification of protein levels of EMT markers, E-Cadherin, Snail, Slug, and

Vimentin in MHCC97L **(A)** and Hep3b **(B)** cells in Figure 5G，H. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

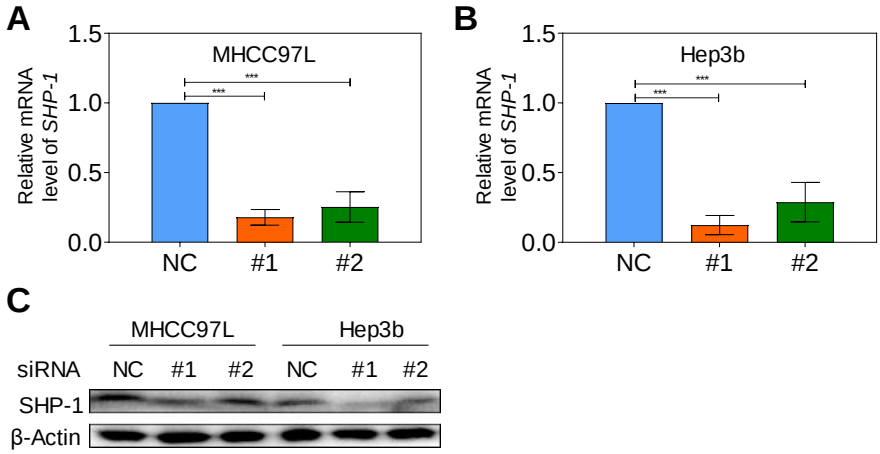

Figure S9. mRNA level of SHP-1 (A, B) and protein level of SHP-1 (C) after knockdown of SHP-1 by siRNA NC，siRNA #1, or siRNA #2. ***, $P < 0.001$.

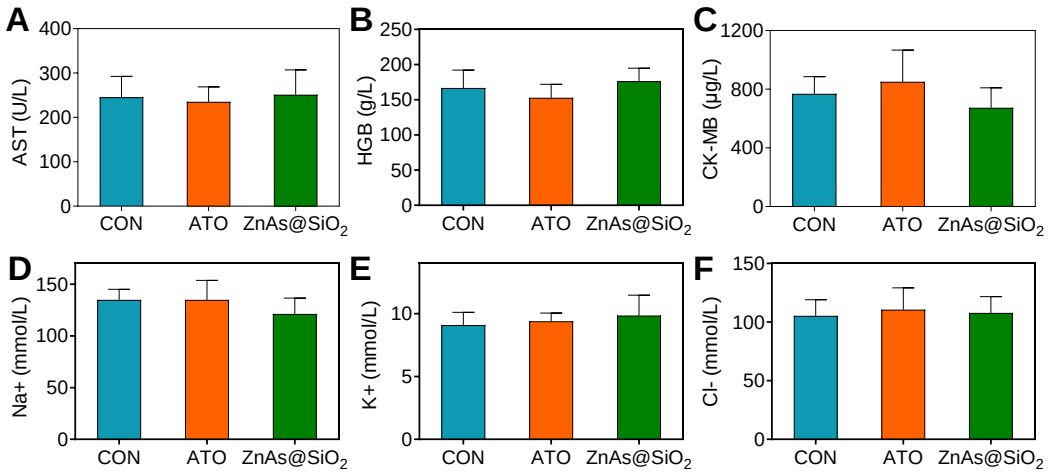

Figure S10. Analysis of blood ALT **(A)**, AST **(B)**, Cr **(C)**, HGB **(D),** and concentration of Na^+ **(E)**, K^+ **(F)** after injections of PBS, ATO, or ZnAs@SiO_2 NPs for 21 days.

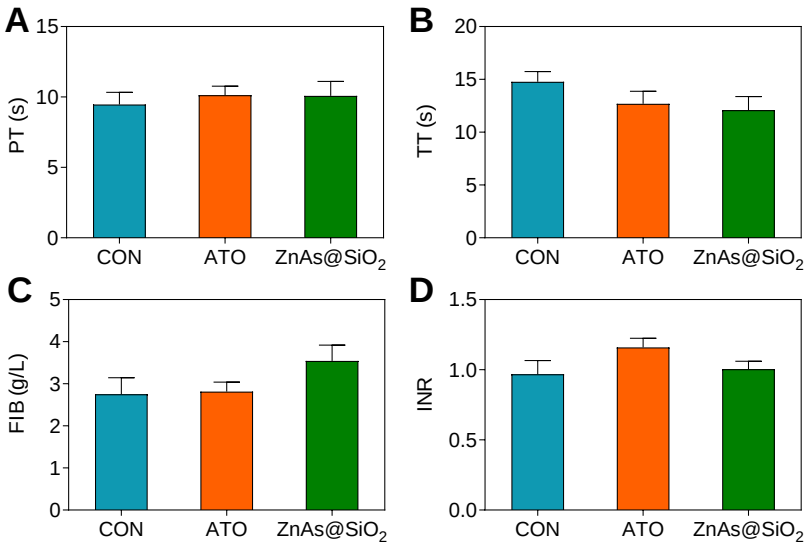

Figure S11. Analysis of blood PT **(A)**, TT **(B)**, FIB **(C)**, and INR **(D)** after injections of PBS, ATO, or ZnAs@SiO_2 NPs for 21 days.

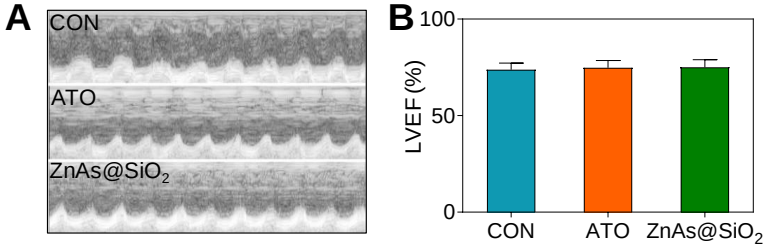

Figure S12. Echocardiography evaluation **(A)** and quantification of LVEF **(B)** after injections of PBS, ATO, or ZnAs@SiO_2 NPs for 21 days.

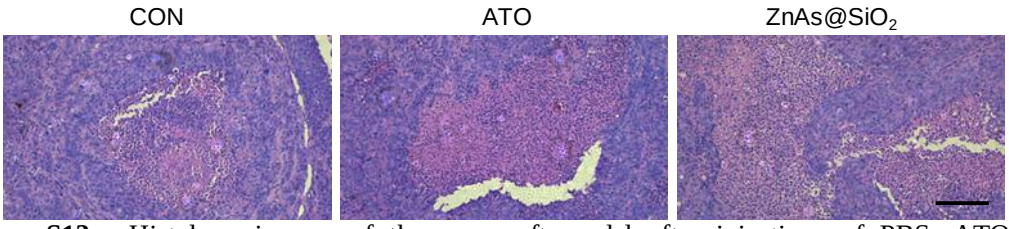

Figure S13. Histology images of the xenograft model after injections of PBS, ATO, or ZnAs@SiO_2 NPs for 21 days. Scale bar, 200 μm.

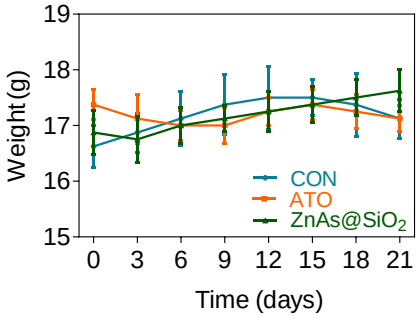

Figure S14. Tumor weight change curves of mice after treatment by PBS, ATO, or ZnAsO@SiO_2.

Table S1. Primer sequences for RT-qPCR assay

Gene		Sequence	Length (bp)
Ki67	Forward	ACGCCTGGTTACTATCAAAAGG	22
	Reverse	CAGACCCATTTACTTGTGTTGGA	23
PCNA	Forward	CCTGCTGGGATATTAGCTCCA	21
	Reverse	CAGCGGTAGGTGTCGAAGC	19
PROM 1 (CD133)	Forward	AGTCGGAAACTGGCAGATAGC	21
	Reverse	GGTAGTGTTGTACTGGGCCAAT	22
Sox-2	Forward	GCCGAGTGGAAACTTTTGTCG	21
	Reverse	GGCAGCGTGTACTTATCCTTCT	22
Oct-4	Forward	CTGGGTTGATCCTCGGACCT	20
	Reverse	CCATCGGAGTTGCTCTCCA	19
CDH1 (E-Cadherin)	Forward	CGAGAGCTACACGTTCACGG	20
	Reverse	GGGTGTCGAGGGAAAAATAGG	21
Slug	Forward	CGAACTGGACACACATACAGTG	22
	Reverse	CTGAGGATCTCTGGTTGTGGT	21
Snail	Forward	TCGGAAGCCTAACTACAGCGA	21
	Reverse	AGATGAGCATTGGCAGCGAG	20
Vimentin	Forward	GACGCCATCAACACCGAGTT	20

	Reverse	CTTTGTCGTTGGTTAGCTGGT	21
β-Actin	Forward	CATGTACGTTGCTATCCAGGC	21
	Reverse	CTCCTTAATGTCACGCACGAT	21
PTPN6 (SHP-1)	Forward	GGAGAAGTTTGCGACTCTGAC	21
	Reverse	GCGGGTACTTGAGGTGGATG	20

Table S2. Sequences for Si-SHP-1

Sequence number	Sequence
si-SHP-1 #1	GCAAGAACCGCTACAAGAA
si-SHP-1 #2	GCACCATCATCCACCTCAA

Reference:

1. Francavilla C, Lupia M, Tsafou K, Villa A, Kowalczyk K, Rakownikow Jersie-Christensen R, et al. Phosphoproteomics of Primary Cells Reveals Druggable Kinase Signatures in Ovarian Cancer. Cell Rep. 2017; 18: 3242-56.