SUPPLEMENTARY DATA

Oral siRNA Delivery to Treat Colorectal Liver Metastases

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SUPPLEMENTARY DATA

SUPPLEMENTARY TABLES

Table S1. Conjugation efficacy of siRNA to AuNP (AR). Molar ratio of FITC-siRNA used to quantify conjugation of siRNA to AuNP. AR100 was optimized and used for further studies. (mean ± SD, N=3)

Sample name	Feed ratio (AuNP : siRNA)	Conjugation ratio (AuNP : siRNA)
AuNP	1 : 0	1 : 0
AR10	1 : 10	1 : 7.80 ± 0.88
AR50	1 : 50	1 : 13.08 ± 0.84
AR100	1 : 100	1 : 32.37 ± 0.936

Table S2 Characterization of TCA-modified glycol chitosan (GT). TNBSA assay was used to quantify the conjugation of TCA to glycol chitosan (GC). The zeta potential values of glycol chitosan after conjugating with different concentration of TCA.

Sample name	Feed ratio (GC : TCA)	Conjugation ratio (GC : TCA)	Zeta potential (mV)
Glycol chitosan	1 : 0	None	45.90 ± 0.10
GT50	1 : 50	1 : 42.80 ± 0.35	29.51 ± 1.70
GT100	1 : 100	1 : 94.77 ± 0.22	0.44 ± 0.37

Table S3 Particles size analysis of nanoparticles. The nanoparticle size and PDI of AuNP, AR, AR-chitosan, AR-GT50 and AR-GT100 was measured by DLS.

Sample name	Particle Diameter (nm)	PDI
AuNP	18.3 ± 1.5	0.37 ± 0.05
AR	22.0 ± 0.5	0.36 ± 0.05
AR-chitosan	58.5 ± 9.7	0.36 ± 0.02
AR-GT50	99.3 ± 3.2	0.37 ± 0.04
AR-GT100	114.8 ± 1.7	0.33 ± 0.04

Table S4 *In vivo* metastasis score of the mice treated with AR-GT100 NPs. Liver metastasis score of the mice treated with saline (control) and AR-GT100 NP. (N=4)

Group	Metastasis score
Control	3.25 ± 0.47
AR-GT100	1.50 ± 0.28

SUPPLEMENTARY DATA

SUPPLEMENTARY FIGURES

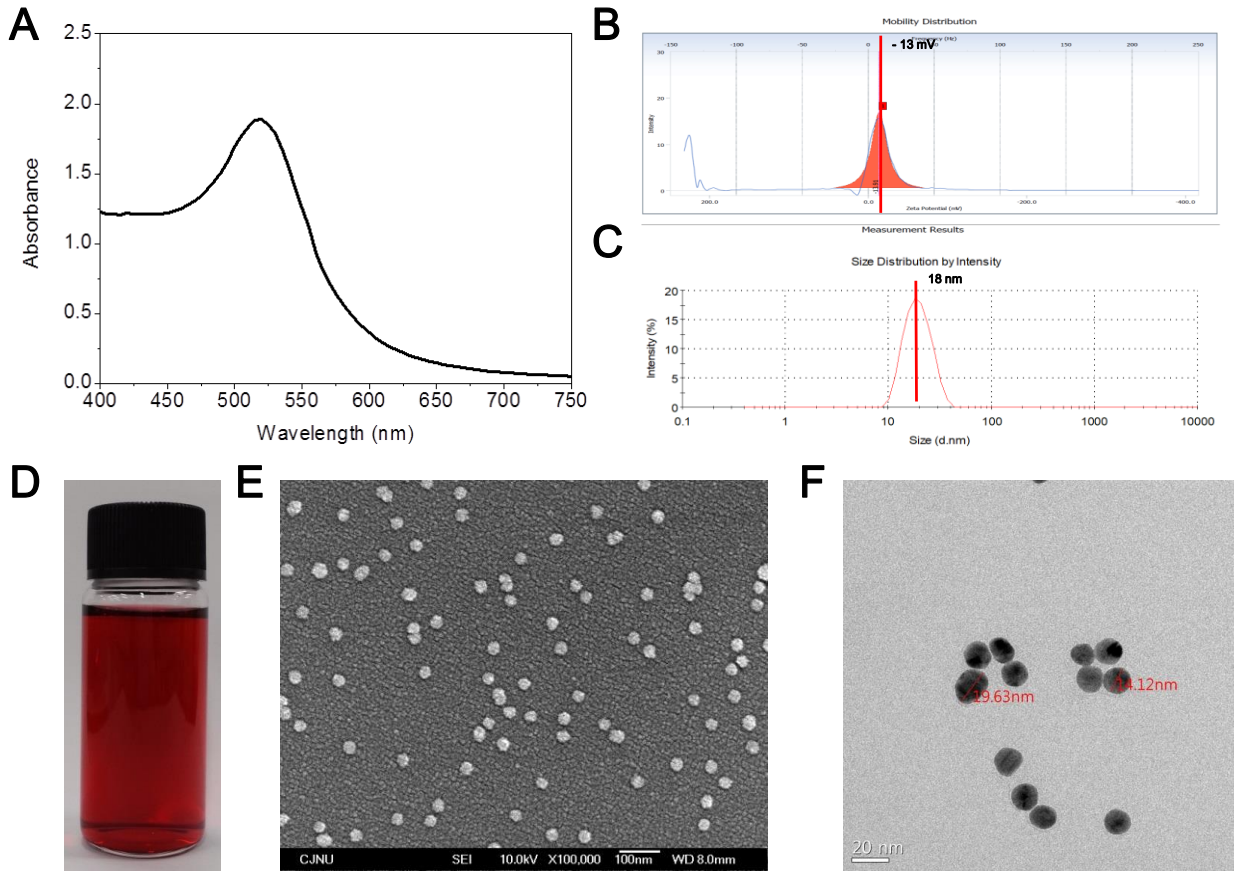

Figure S1. Synthesis and characterization of gold nanoparticles (AuNP). (A) UV-vis spectrum (at 520 nm) **(B)** Surface zeta potential (negative charge: -13 mV) **(C)** DLS spectra (average: 20.17 nm) **(D)** Digital photograph (color: red wine) **(E)** SEM image (approximately 20 nm) and **(F)** TEM image (approximately 20 nm) of AuNP.

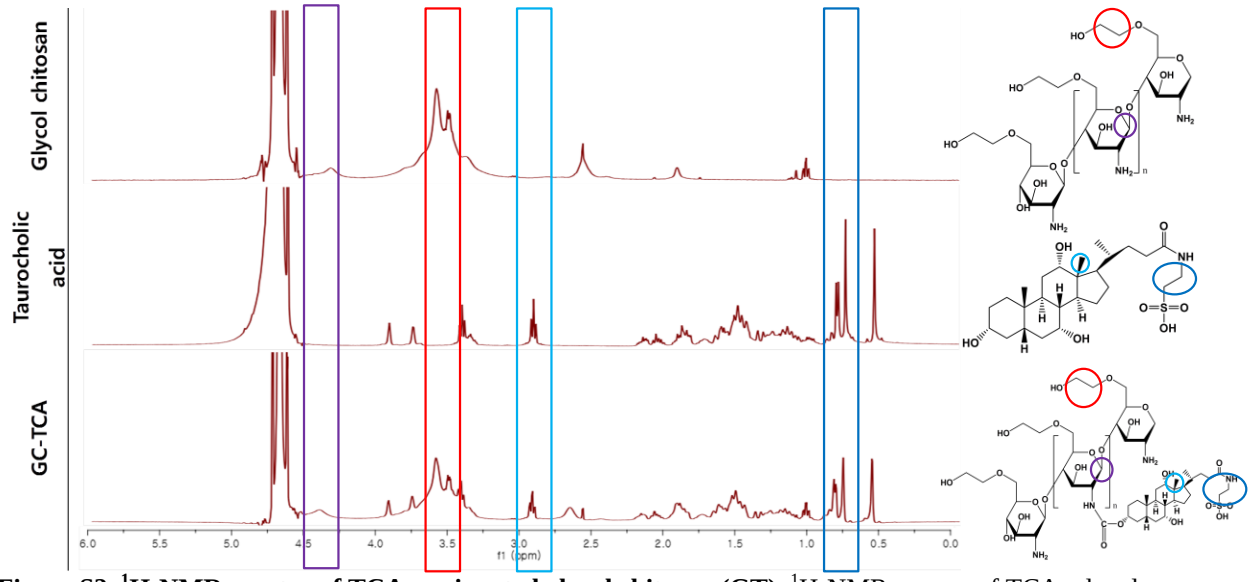

Figure S2. ^{1}H-NMR spectra of TCA-conjugated glycol chitosan (GT). ^{1}H-NMR spectra of TCA, glycol chitosan, and TCA-modified glycol chitosan (GT). The assignments of the relevant proton signals of TCA and glycol chitosan are marked in color coded rectangular and circular boxes.

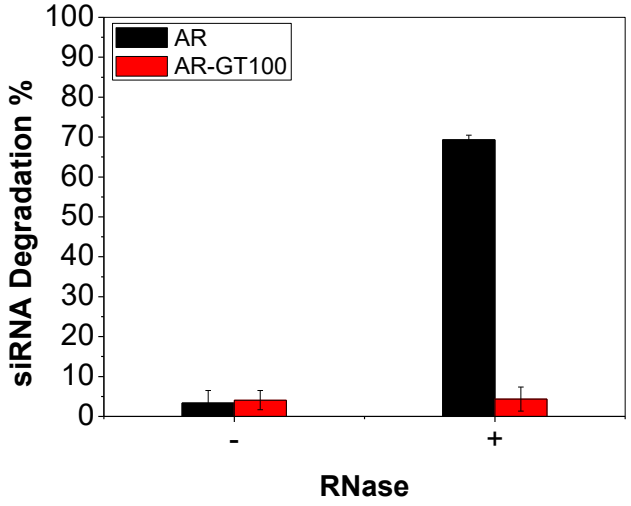

Figure S3. Stability of siRNA in the presence of biological enzymes. FITC conjugated AKT siRNA were loaded in AR and AR-GT100 and incubated with RNase (1 unit/μg of siRNA) for 60 min. The enzymatic degradation was quantified by the gain in the fluorescence intensities after incubating the AR and AR-GT100 with RNase.

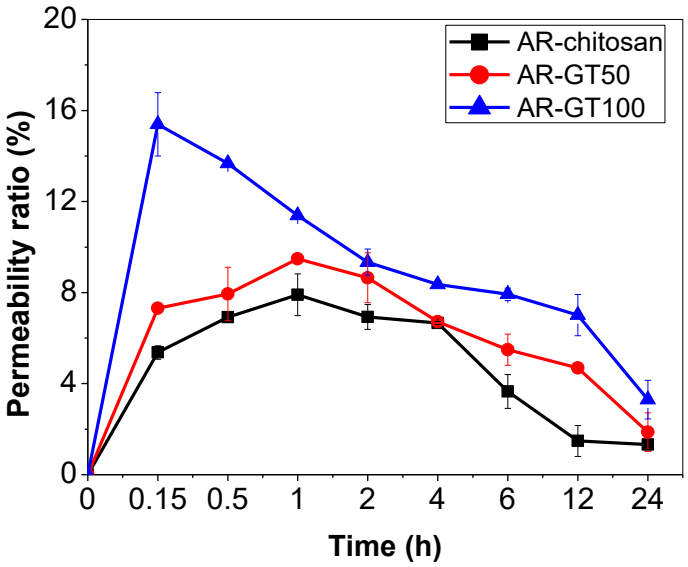

Figure S4. Time-dependent Caco-2 cell transwell permeability study. TCA-mediated nanoparticle permeability through the Caco-2 cell monolayer. The permeability ratio (%) of the nanoparticles was obtained by examining cell migration in the apical to basolateral direction for 24hr.

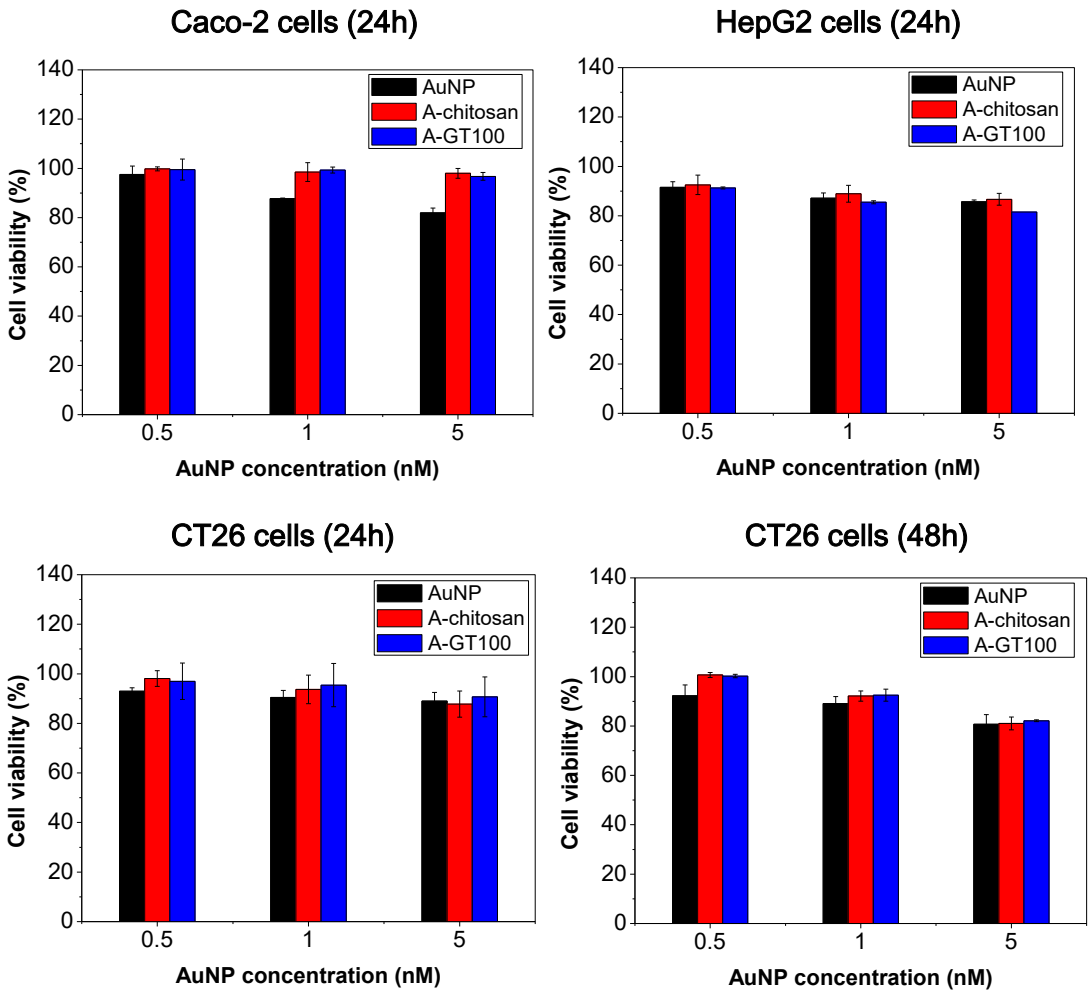

Figure S5. *In vitro* cellular toxicity. Cellular toxicity study of siRNA carrier vehicle at 24 h and 48 h in Caco-2, HepG2 and CT26 cell lines.

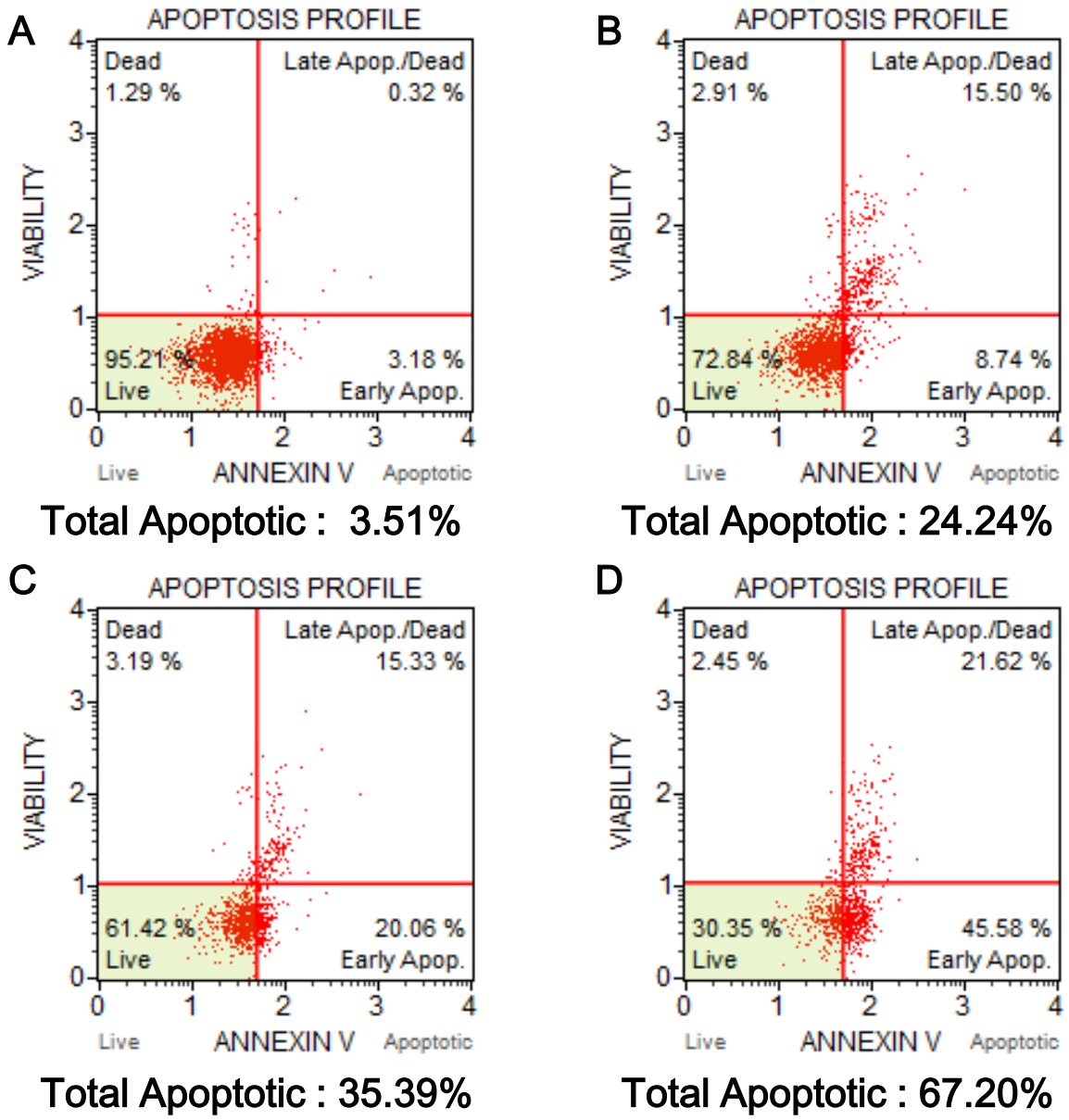

Figure S6. *In vitro* Apoptosis analysis. Annexin V & dead cell marker assay was used to study the induction of apoptosis in the HepG2 cell line treated with **(A)** PBS (control), **(B)** free Akt-siRNA, **(C)** AR-chitosan and **(D)** AR-GT100.

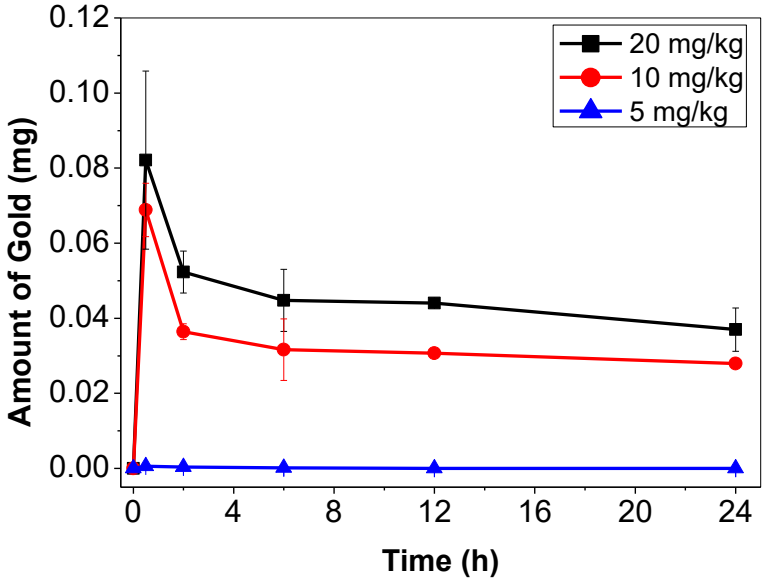

Figure S7. Measurement of the dose-dependent Au accumulation in the liver. Quantification of the accumulation of Au ions in liver tissue treated with different doses of AR-GT100 (0.2, 1.0, 1.5 mg/mice) during a 0.5 to 24h time line (n=4).

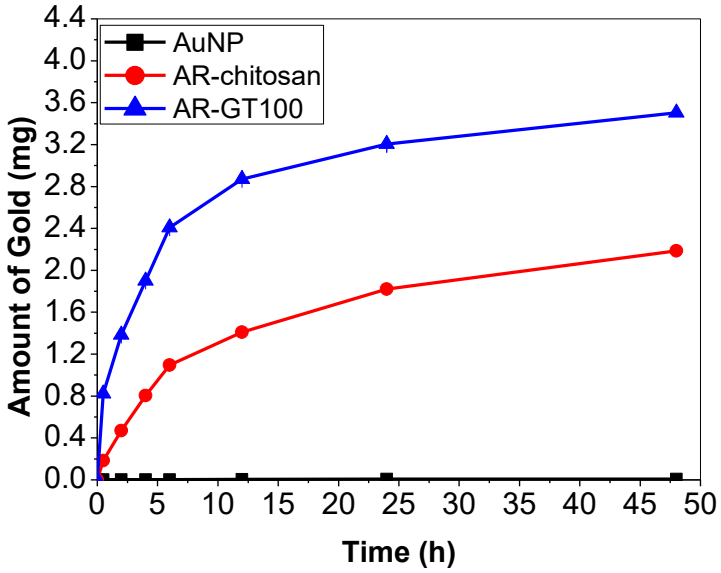

Figure S8. Accumulation of AuNP, AR-chitosan and AR-GT100 in the liver. Quantitative analysis of the Au ions accumulation in the liver tissue. Mice were treated with bare AuNP, AR-chitosan and AR-GT100 and the gold nanoparticles accumulation was monitored during 0.5 to 48 h (n=4).

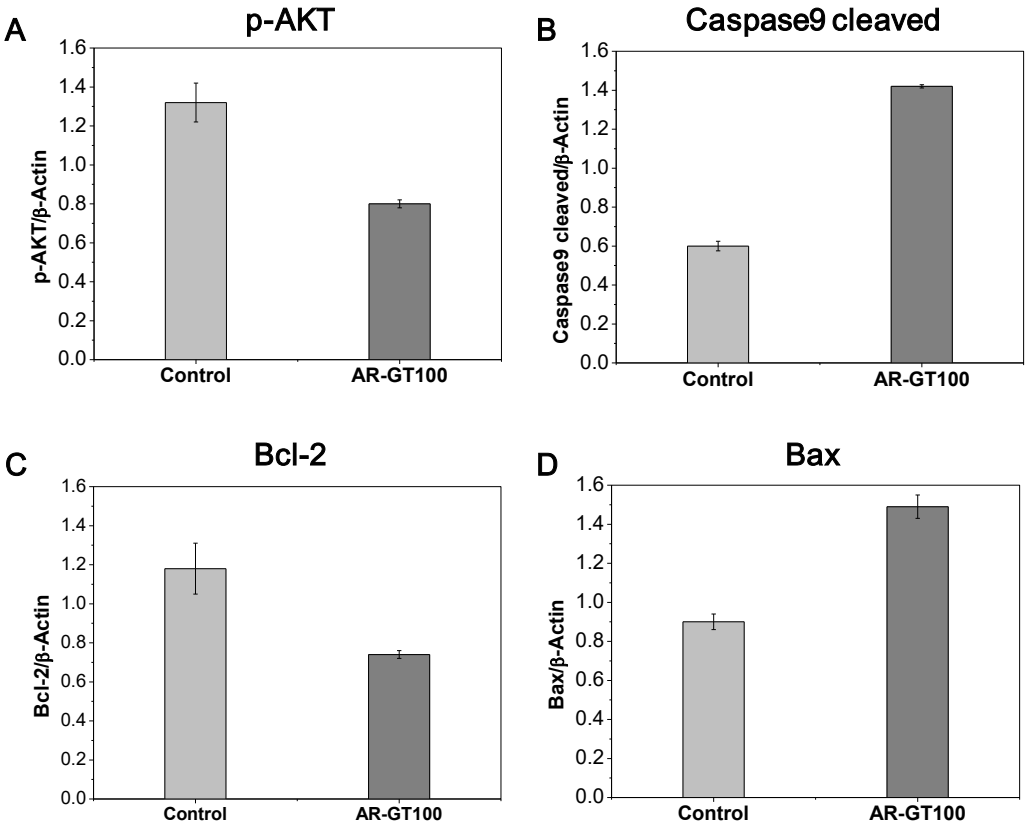

Figure S9. *In vivo* mechanism of AR-GT100 transport. Quantitative analysis of different protein (p-AKT, Caspase9 cleaved, Bcl-2 and Bax) in control and AR-GT100 treated animals.