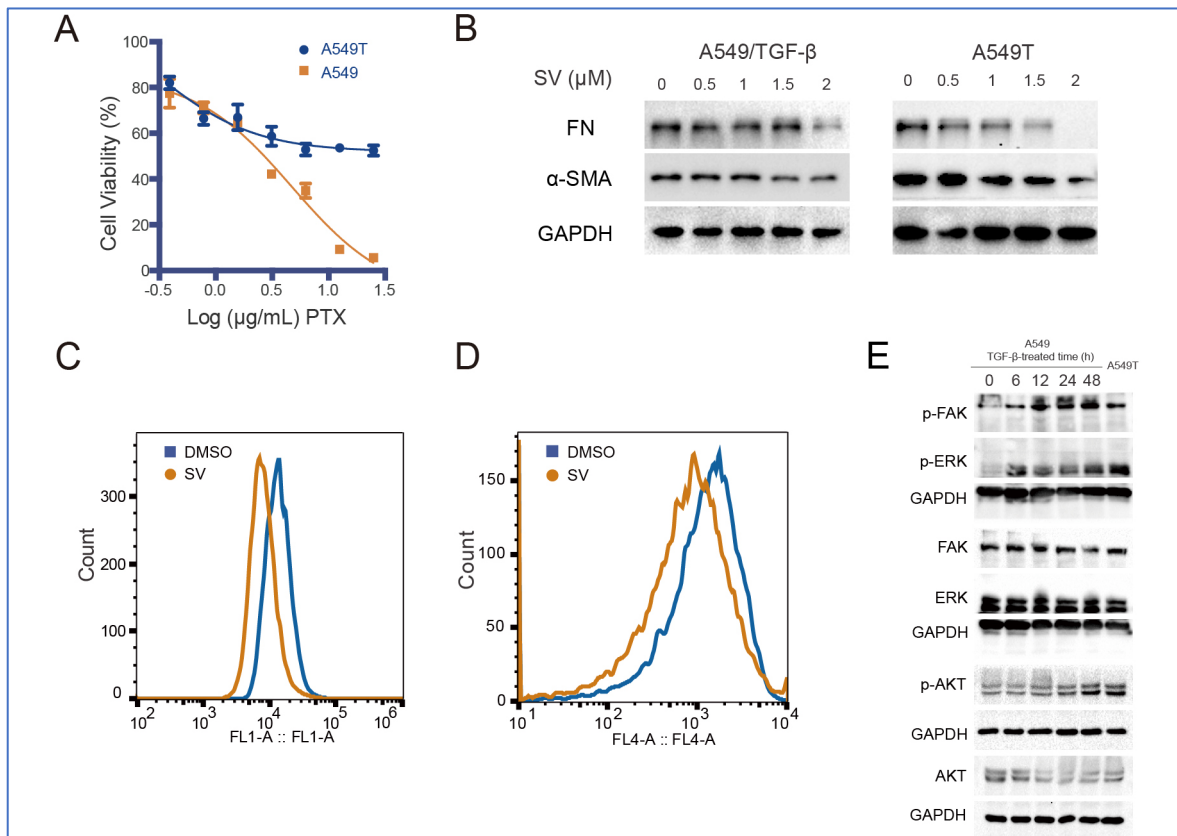


# Targeting Lipid Metabolism to Overcome EMT-Associated Drug Resistance Via Integrin $\beta 3$ /FAK Pathway and Tumor-Associated Macrophage Repolarization Using Legumain-Activatable Delivery

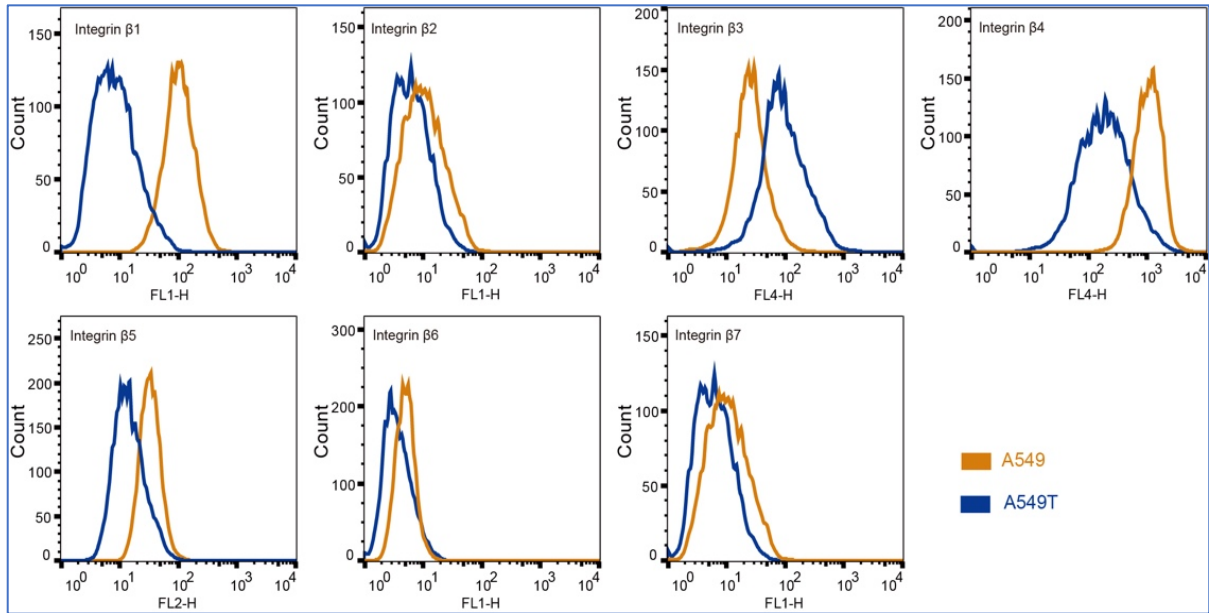
Hongyue Jin <sup>1,2</sup>, Yang He <sup>1,2</sup>, Pengfei Zhao <sup>1</sup>, Ying Hu <sup>3,\*</sup>, Jin Tao <sup>3</sup>, Jiang Chen <sup>4</sup>, Yongzhuo Huang <sup>1,2,\*</sup>

## Supplementary Figures

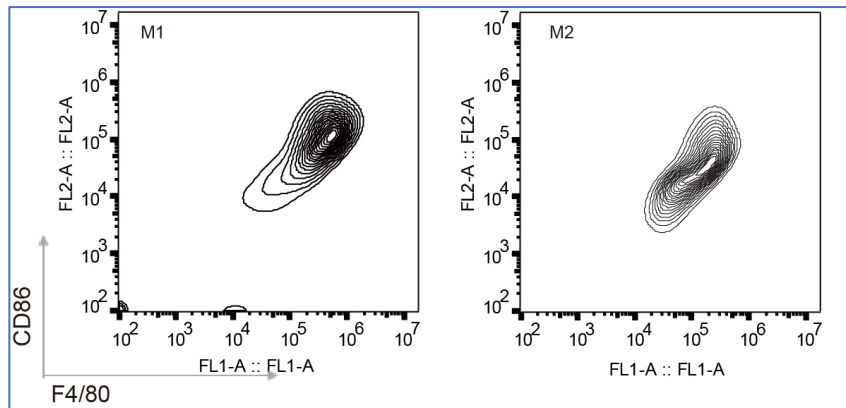


**Figure S1** (A) Cell viability in A549T and A549 cells with PTX treatment. (B) Western blot assay of the mesenchymal markers. (C) Flow cytometry analysis of CTB-488-labeled lipid rafts in A549T cells. (D) Downregulation of integrin  $\beta 3$  in A549T cells by SV treatment. (E) The phosphorylated FAK/ERK was up-regulated in the TGF- $\beta$ -induced, mesenchymal-type A549 cells; the right lane was the A549T cells with innate EMT nature, showing the activation of FAK/ERK, too.

## Supporting Information

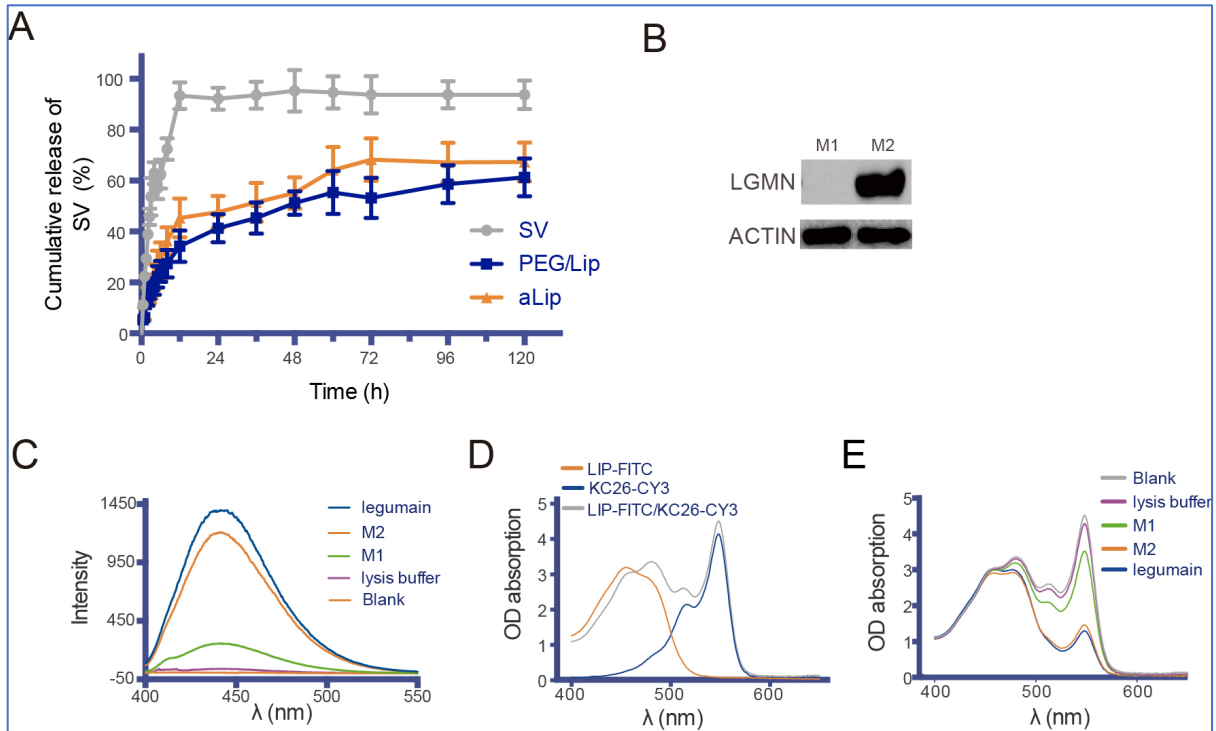


**Figure S2** Flow cytometry analysis of integrins in A549 and A549T cells. The EMT-type A549T was characterized by the up-regulation of Integrin  $\beta$ 3 expression. Other integrin variants in A549T cells were much lower than those in the A549 cells.

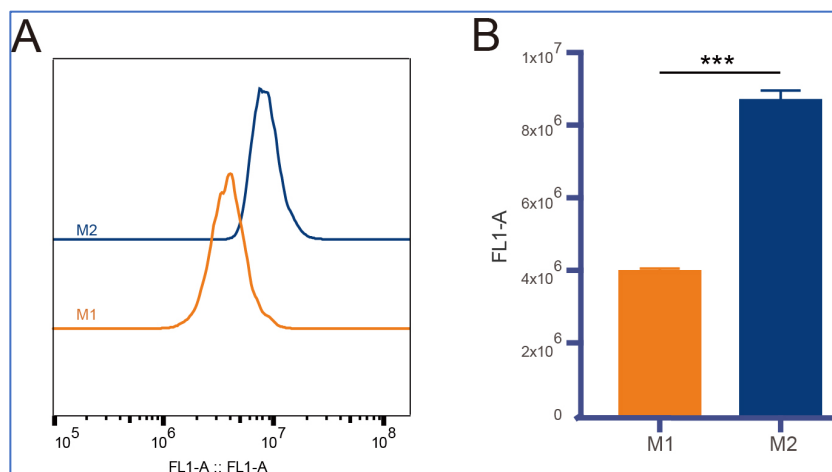


**Figure S3** Flow cytometry analysis of BMDM-induced M1 $\Phi$  and M2 $\Phi$ .

## Supporting Information

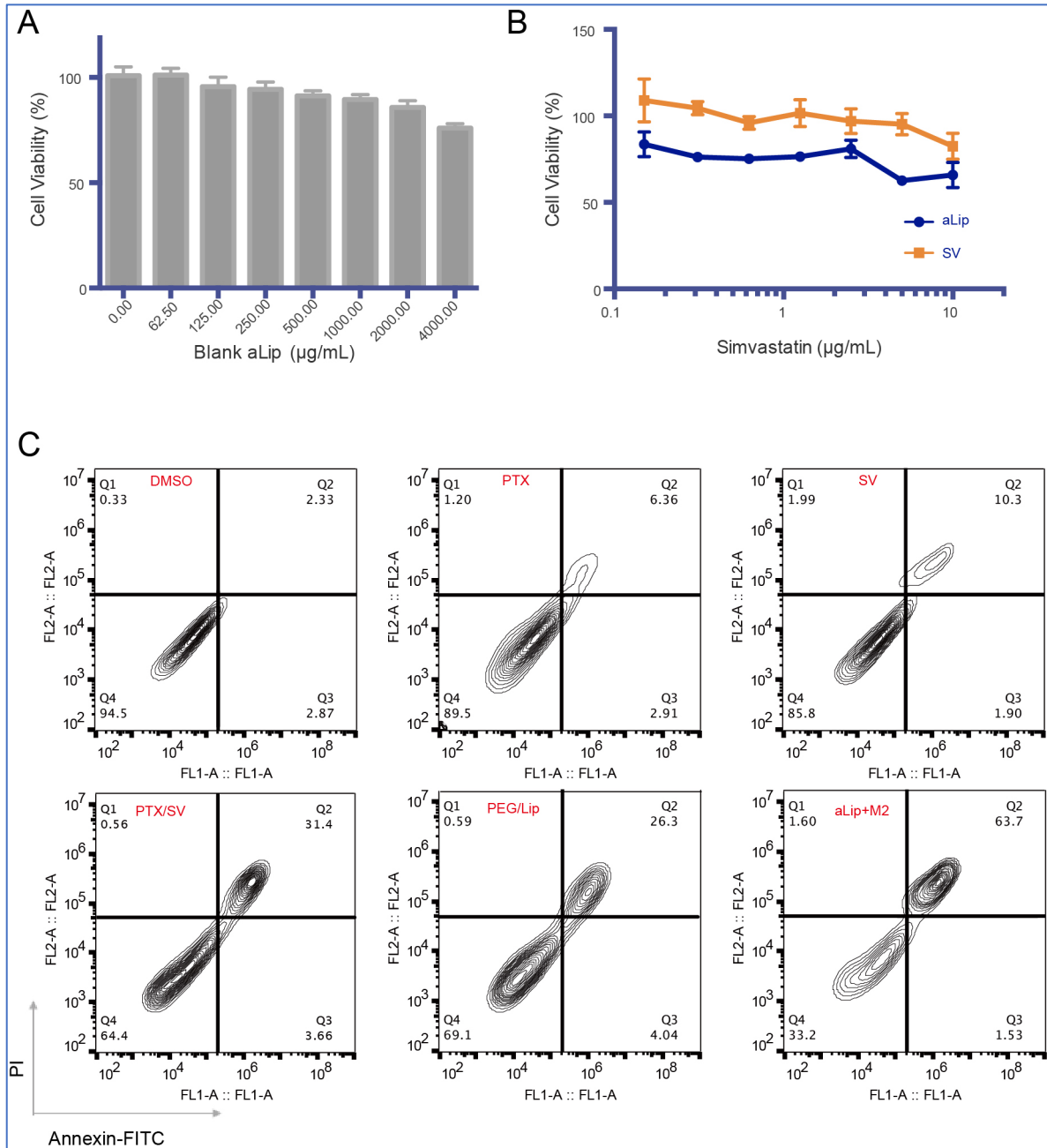


**Figure S4** (A) In vitro release of SV. (B) Legumain expression in M1Φ and M2Φ. (C) Legumain activity in M1Φ and M2Φ was measured by the AAN-AMC probe. (D) The aLip dual labeled with DSPE-PEG-FITC and DSPE-PEG-KC26-CY3 displays two characteristic absorption peaks of FITC and Cy3. (E) After treatment with the M2Φ lysates, the absorbance spectrum shows the intensity of Cy3 reduced, due to the cleavage of the linker KC26 and the consequent detachment of Cy3, but FITC spectra remained the same due to the non-cleavable DSPE-PEG-FITC.



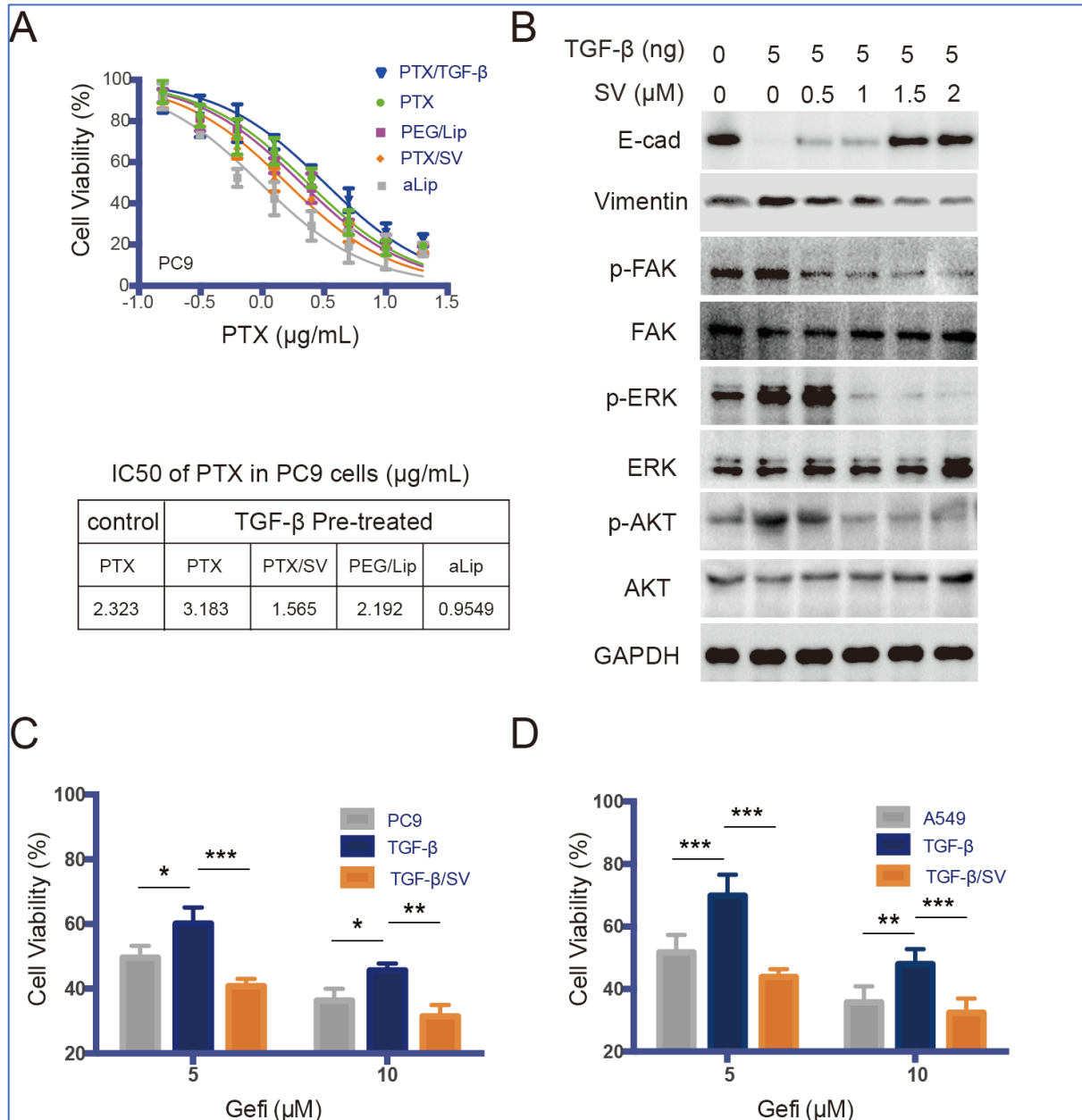
**Figure S5** Uptake of aLip in the BMDM-induced M1Φ and M2Φ. (A) Flow cytometry analysis of M1Φ and M2Φ. (B) Quantitative analysis of FACS results.

## Supporting Information



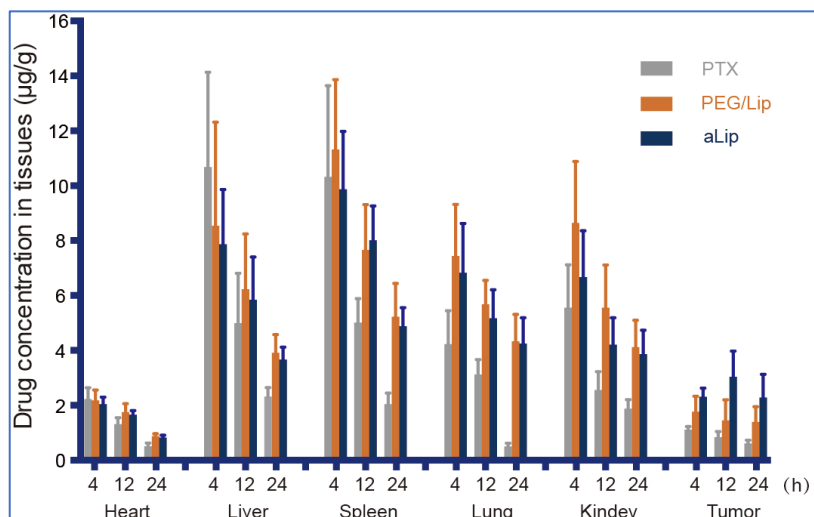
**Figure S6** (A) The cytotoxicity of the blank aLip in M2Φ. (B) The cytotoxicity of free SV and the drug-loaded aLip in in M2Φ. (C) Cell apoptosis assay of free drugs and aLip.

## Supporting Information

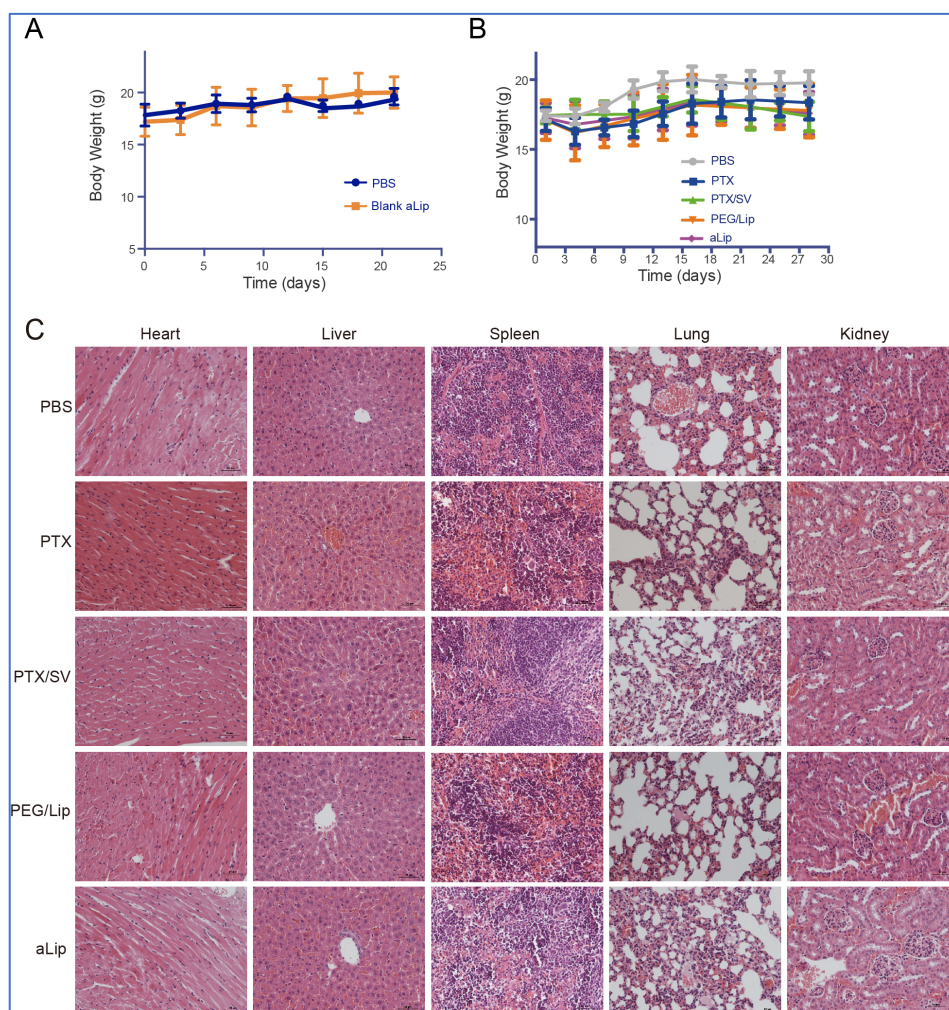


**Figure S7** The antitumor efficacy in PC9 cells and the mechanism study. (A) The drug resistance was developed in the PC9 cells treated with TGF-β. (B) EMT was induced by exposure to TGF-β. However, SV treatment can reverse EMT and repolarize the cells back to epithelial type from the mesenchymal, as characterized by the increased E-cad and reduced vimentin. Meanwhile, the TGF-β-induced FAK/ERK/AKT phosphorylation was suppressed by SV treatment. The TGF-β-induced mesenchymal PC9 (C) and A549 (D) showed drug resistance to gefitinib but was resensitized by SV.

## Supporting Information

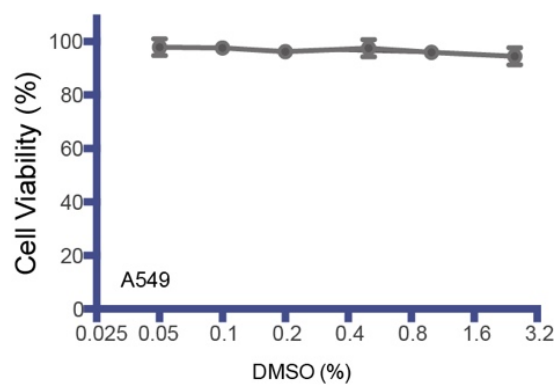


**Figure S8** Biodistribution study of the aLip by determining PTX concentration.



**Figure S9** Preliminary biosafety evaluation. (A) The biocompatibility of the blank aLip. (B) Body weight curve during the treatment. (C) Histological examination of the major organs after treatment in the A549T tumor-bearing mice.

## Supporting Information



**Figure S10** The cytotoxicity test of the solvent DMSO. No cytotoxicity was found at a concentration up to 3%. A safe concentration of 0.2% DMSO was used for the cellular studies.

## Supporting Information

### Supplementary Tables

**Table S1** The IC<sub>50</sub> values measured by MTT test.

	A549T	A549	TGF-β induced A549
PTX (μg/mL)	8.19	2.31	5.28
PTX/SV (μg/mL)	1.82	2.10	2.01
SV (μg/mL)	7.9	11.4	9.2

**Table S2** RT-PCR primers.

Name	Primer	Sequence	Size
Mus GAPDH	Forward	5'- ATGGGTGTGAACCACGAGA -3'	229 bp
	Reverse	5'- CAGGGATGATGTTCTGGGCA -3'	
Mus IL-1b	Forward	5'- TCAGGCAGGCAGTATCACTC -3'	250 bp
	Reverse	5'- AGCTCATATGGGTCCGACAG -3'	
Mus IFN-γ	Forward	5'- CGCTACACACTGCATCTTGG -3'	174 bp
	Reverse	5'- TCCTTTTGCCAGTTCCTCCA -3'	
Mus IL-1a	Forward	5'- TGAAGAAGAGACGGCTGAGT -3'	159 bp
	Reverse	5'- CAAACTTCTGCCTGACGAGC -3'	
Mus CCL-17	Forward	5'- AGAGTGCTGCCTGGATTA -3'	110 bp
	Reverse	5'- GGACAGTCAGAAACACGATG -3'	
Mus Arg1	Forward	5'- ACATCAACACTCCCCTGACA -3'	157 bp
	Reverse	5'- CGCAAGCCAATGTACACGAT -3'	
Mus CD206	Forward	5'- TTGTGGAGCAGATGGAAGGT -3'	200 bp
	Reverse	5'- TCGTAGTCAGTGGTGGTTCC -3'	
Mus CCL-22	Forward	5'- TCTGCTGCCAGGACTACAT -3'	117 bp
	Reverse	5'- CTCGGTTCTTGACGGTTAT -3'	
Mus Il-10	Forward	5'- ACCTGGTAGAAGTGATGCCC -3'	193 bp
	Reverse	5'- ACACCTTGGTCTTGGAGCTT -3'	
Mus TGF-β1	Forward	5'- AATGGTGGACCGCAACAAC -3'	213 bp
	Reverse	5'- CCAAGGTAACGCCAGGAAT -3'	
Mus TNF-α	Forward	5'- CGTCAGCCGATTTGCTATCT -3'	206 bp
	Reverse	5'- CGGACTCCGCAAAGTCTAAG -3'	
Mus ABCA1	Forward	5'- ACCCGCTGTATGGAAGGAAA -3'	250 bp
	Reverse	5'-TCTGAAGGATGTCTGCGGTT -3'	



## Supporting Information

**Table S3** Quantification of protein bands on Fig 1A and S1D using densitometry.

	A549					A549T
	TGF- $\beta$ -treated time (h)					
	0	6	12	24	48	
E-CAD/GAPDH	0.2302	0.1887	0.0702	0.0883	0.0350	0.0164
VIMENTIN/GAPDH	0.2186	0.5834	0.2928	0.3779	0.4216	0.5744
p-FAK/GAPDH	0.1799	0.2041	0.4851	0.5301	0.5829	0.2873
p-ERK/GAPDH	0.1777	0.4724	0.4722	0.7363	0.6562	0.7616
FAK/GAPDH	0.3909	0.4216	0.4371	0.4210	0.3244	0.5433
ERK/GAPDH	1.1927	1.1037	1.1265	1.1542	1.1503	1.1619
p-AKT/GAPDH	0.3274	0.3162	0.3673	0.4046	0.5867	0.7126
AKT/GAPDH	0.4266	0.3960	0.2887	0.3228	0.3209	0.3126

**Table S4** Quantification of protein bands on Fig 1E using densitometry.

SV ( $\mu$ M)		0	0.5	1	1.5	2	5
A549/TGF- $\beta$	E-CAD/GAPDH	0.2154	0.4044	0.4108	0.5042	0.6256	0.5374
	vimentin/GAPDH	5.6885	8.8112	8.5935	3.3216	2.5216	1.8908
A549T	E-CAD/GAPDH	0.0040	0.0336	0.0552	0.1462	0.2243	0.1925
	vimentin/GAPDH	0.2796	0.2510	0.1623	0.2388	0.1365	0.0901

**Table S5** Quantification of protein bands on Fig 1H using densitometry.

SV ( $\mu$ M)		0	0.5	1	1.5	2
A549/TGF- $\beta$	p-FAK/GAPDH	0.4897	0.2621	0.2825	0.3559	0.1398
	p-ERK/GAPDH	0.9524	0.7923	0.7003	0.7824	0.1731
	FAK/GAPDH	0.7196	0.6567	0.9229	0.6989	0.6411
	ERK/GAPDH	0.9050	1.0342	1.2375	0.8814	1.1212
	p-AKT/GAPDH	1.2653	1.1817	0.8387	0.8252	0.8359
	AKT/GAPDH	2.3602	2.2881	2.5054	2.6453	2.3689
A549T	p-FAK/GAPDH	0.7742	0.9273	0.5137	0.3323	0.2736
	p-ERK/GAPDH	0.5531	0.9290	0.5401	0.3804	0.3122
	FAK/GAPDH	0.9443	0.8529	0.9000	1.2075	0.9897
	ERK/GAPDH	1.7087	1.1479	0.8330	1.2102	1.6117
	p-AKT/GAPDH	1.1151	0.8512	0.9377	0.6288	0.8120
	AKT/GAPDH	1.0569	1.1378	1.0588	0.8321	0.7922