Electronic Supplementary Information

Synergy of Hypoxia Relief and Chromatin Remodeling to Overcome Tumor Radiation Resistance

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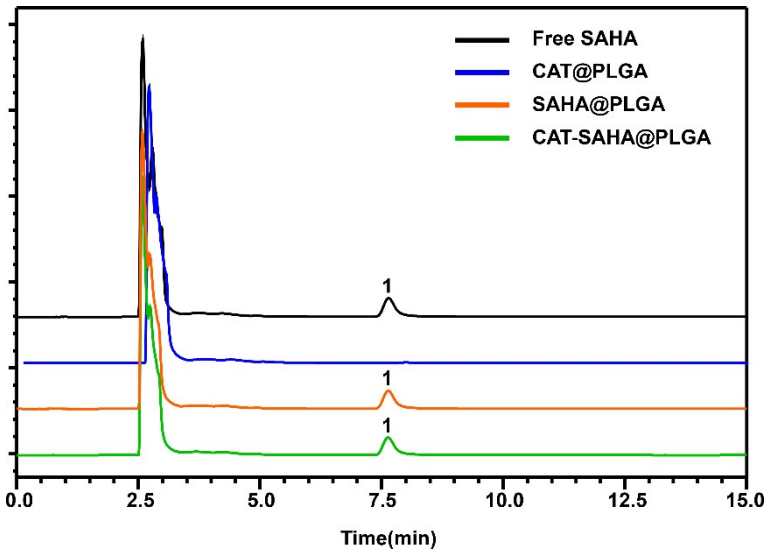

Figure S1. High Performance Liquid Chromatography (HPLC) data of Free SAHA (Black), CAT@PLGA (Blue), SAHA@PLGA (Orange) and CAT-SAHA@PLGA (Green). The peak 1 belongs to SAHA.

Table.S1 The encapsulation efficiency (EE) of catalase.

	Total amount of catalase (mg)	**Amount of catalase in the supernatant (mg)**	**The encapsulation efficiency (%)**
CAT-SAHA@PLGA-1		2.77	
CAT-SAHA@PLGA-2	8	2.54	66.88 ± 1.44
CAT-SAHA@PLGA-3		2.64	
CAT@PLGA-1		2.35	
CAT@PLGA-2	8	2.42	69.50 ± 1.22
CAT@PLGA-3		2.55	

* Data was shown as mean ± SD (n = 3).

Table.S2 The encapsulation efficiency (EE) of SAHA.

	Total amount of SAHA (mg)	**Amount of SAHA loaded in nanoparticles (mg)**	**The encapsulation efficiency (%)**
CAT-SAHA@PLGA-1		0.99	
CAT-SAHA@PLGA-2	5	0.91	18.78 ± 0.92
CAT-SAHA@PLGA-3		0.92	
SAHA@PLGA-1		0.96	
SAHA@PLGA-2	5	1.14	21.49 ± 2.07
SAHA@PLGA-3		1.13	

* Data was shown as mean ± SD (n = 3).

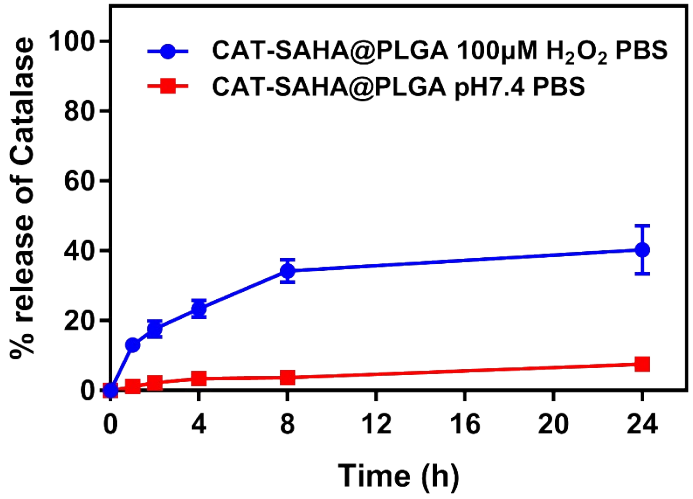

Figure S2. In vitro release profile of catalase from CAT-SAHA@PLGA nanoparticles incubated with or without 100 μM H_2O_2 in PBS (pH 7.4).

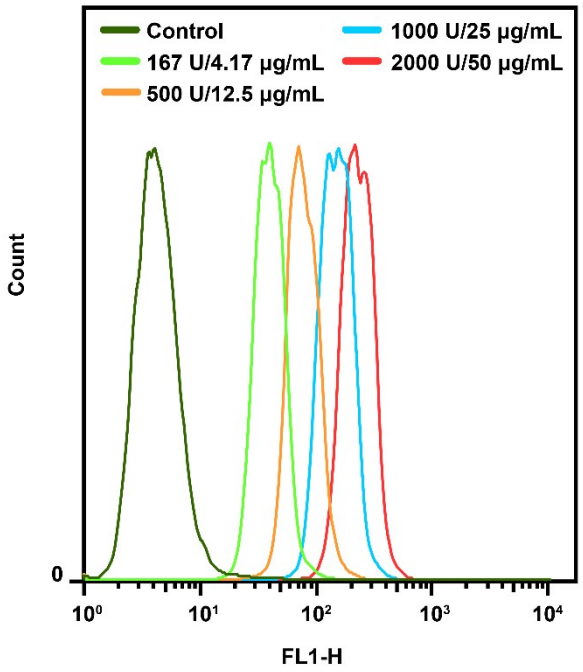

Figure S3. Flow cytometry of CT26 cells incubated with different concentrations of CAT-SAHA@PLGA (CAT/SAHA = 0 U/0 μg/mL, 167 U/4.17 μg/mL, 500 U/12.5 μg/mL, 1000 U/25 μg/mL and 2000 U/50 μg/mL).

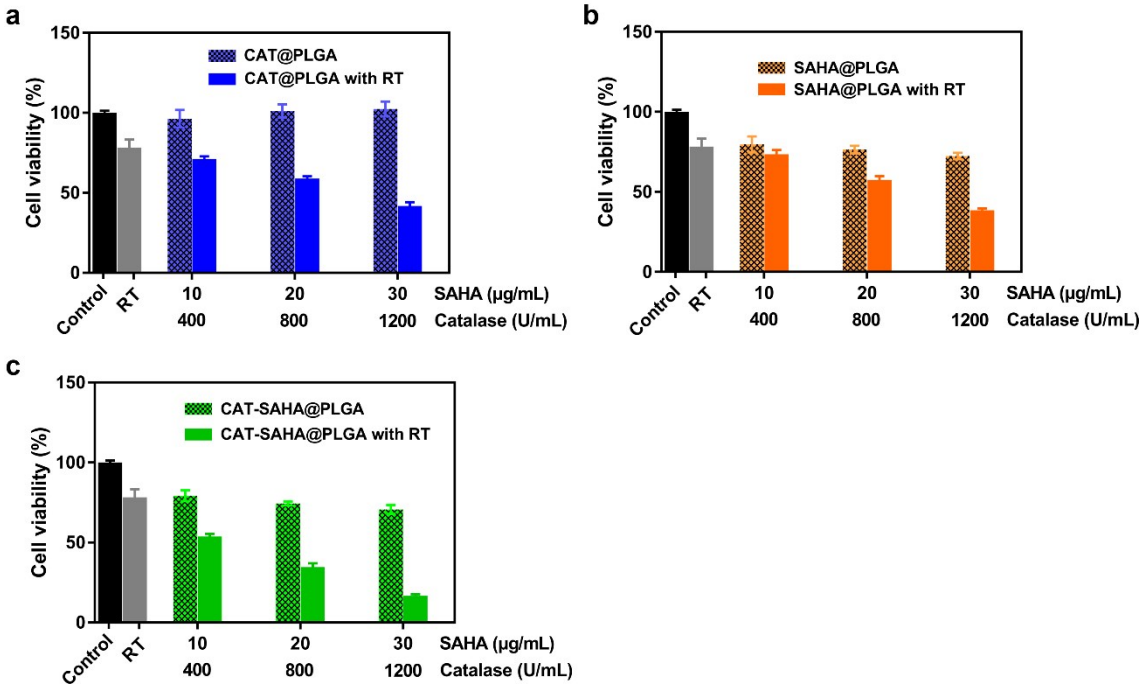

Figure S4. Cytotoxicity of CAT@PLGA, SAHA@PLGA and CAT-SAHA@PLGA of various concentrations in the absence or presence of radiation (6 Gy). (a) Cytotoxicity of CAT@PLGA of various concentrations in the absence or presence of radiation (6 Gy). (b) Cytotoxicity of SAHA@PLGA of various concentrations in the absence or presence of radiation (6 Gy). (c) Cytotoxicity of CAT-SAHA@PLGA of various concentrations in the absence or presence of radiation (6 Gy).

Table.S3 Sensitizer enhancement ratio (SER) of different nanoparticles.

Dose of radiation (Gy)	**% of suppression**			
	Saline	**CAT@PLGA**	**SAHA@PLGA**	**CAT-SAHA@PLGA**
0	0	0	0	0
2	6.4860	18.1643	9.2373	22.1525
6	21.6980	41.8022	24.7262	53.4158
10	36.9100	65.4399	54.0116	84.6792
LD50 (Gy)	16.3330	6.7230	10.2700	4.525
SER		2.4294	1.5904	3.6095

* The median lethal dose (LD50) data were calculated by IBM SPSS v22 software (n = 3).

Table.S4 Combination index (CI) data for combo of catalase and SAHA.

Dose of catalase (U/mL)	**Dose of SAHA (μg/mL)**	**Fraction affected**	**Combination index**
400	10	0.4677	0.7884
800	20	0.2607	0.6399
1200	30	0.1248	0.4002

* The combination index (CI) data and fraction affected data were analyzed by CompuSyn v1.0 software.

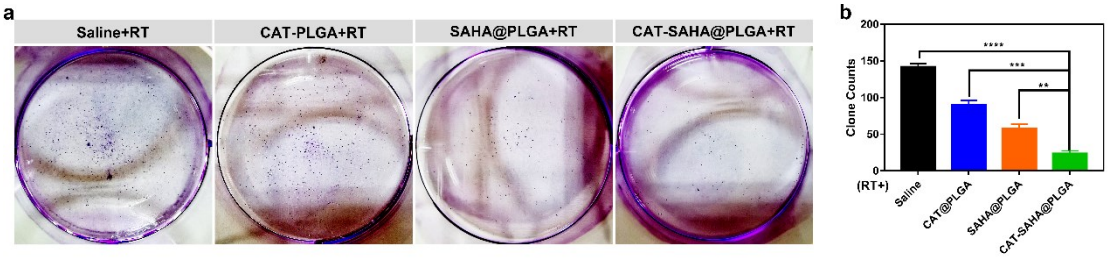

Figure S5. Images and quantification of CT26 cell clones. Data are shown as mean ± SD; **p < 0.01, ***p < 0.001, ****p < 0.0001.

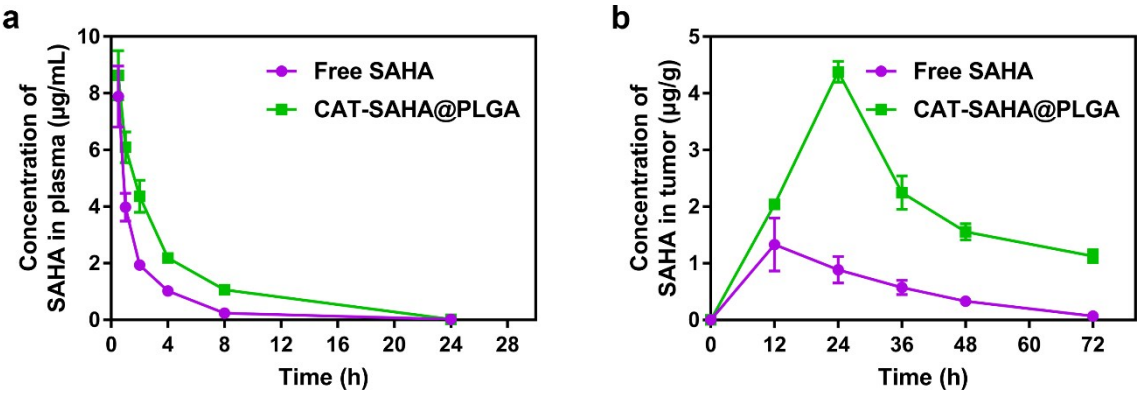

Figure S6. Accumulation and the pharmacokinetic behavior of SAHA. (a) Pharmacokinetic curves of SAHA in vivo (SAHA = 1 mg/kg). Blood samples were collected and analyzed with HPLC at different time points (0.5, 1, 2, 4, 8, 24 h). (b) The dynamic concentrations of SAHA accumulated in the tumor tissues after intravenous injection of free SAHA or CAT-SAHA@PLGA (SAHA = 1 mg/kg). Data are shown as mean ± SEM (n = 3).

Table.S5 Pharmacokinetic parameters.

	AUC_{0-t} (mg/L*h)	$AUMC_{0-t}$ (mg/L*h^2)	MRT_{0-t} (h)	$t_{1/2}$ (h)	C_{max} (mg/L)
Free SAHA	20.58±3.22	45.23±2.84	2.50±0.38	2.60±0.65	7.89±2.15
CAT-SAHA@PLGA	37.46±6.85	135.73±28.65	3.83±0.13	3.95±1.39	8.64±1.74

* Data was shown as mean ± SD (n = 4). The pharmacokinetic parameters of SAHA were analyzed by DAS 2.1.1 software.

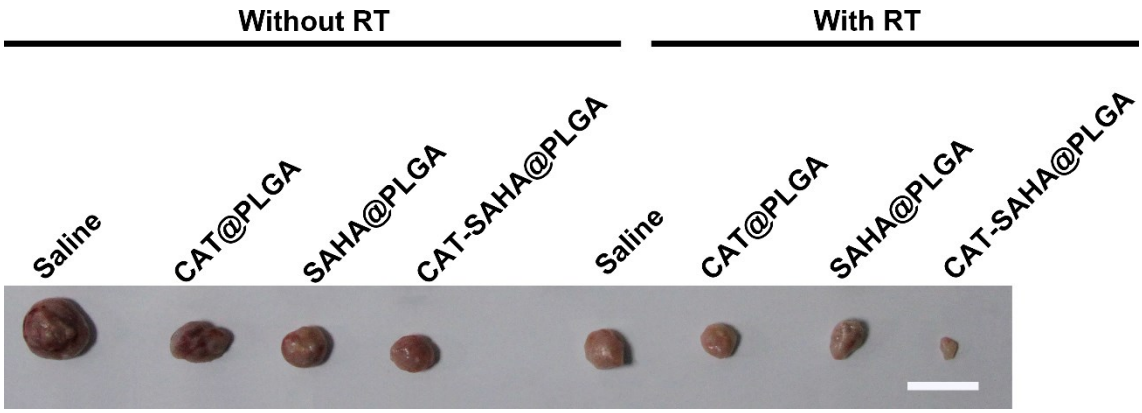

Figure S7. Tumor photos of different groups at day 14, scale bar = 2 cm.

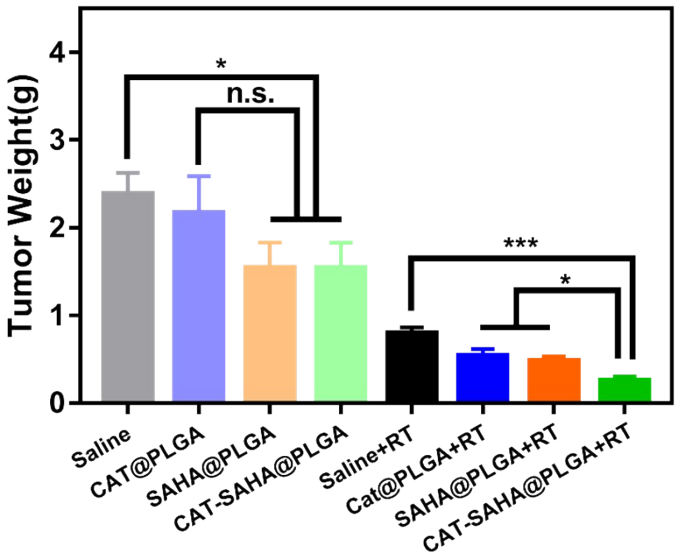

Figure S8. Tumor weight of different groups at day 14.